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Vol. IX January, 1924 No. 1

THE CALCIFEROUS GLANDS OF LUMBRICIDAE AND DIPLOCARDIA

WITH TWELVE PLATES

BY
FRANK SMITH

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STEPHEN ALFRED FORBES
WILLIAM TRELEASE
HENRY BALDWIN WARD

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THE CALCIFEROUS GLANDS OF LUMBRICIDAE AND DIPLOCARDIA

WITH TWELVE PLATES

BY

FRANK SMITH

Contributions from the
Zoological Laboratory of the University of Illinois
No. 233

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INTRODUCTION

Certain organs related to the posterior part of the esophagus in earthworms belonging to the family Lumbricidae, and also in those of certain other systematic groups, have been studied and described at various times since the early part of the nineteenth century. These organs have received various names, of which calciferous glands, esophageal glands, and glands of Morren are among those more commonly used in the recent literature on the subject. The purpose in the preparation of this paper has been to bring together the more important results of the work of previous investigators who have studied the anatomy of such organs in the Lumbricidae; to add some facts gained from my own study of worms of this group; and to describe similar organs found in two new species of the genus Diplocardia which belongs to another family of earthworms. This involves the description of these two new species, but it is hoped that a better basis for an outline of the probable history of the development of the complicated structures in the Lumbricidae will result from the discussion of the interesting series of such organs found in the different species of Diplocardia. Among these species we find organs of a degree of complexity similar to that in Lumbricidae; others of a very simple type in which we have merely a few longitudinal folds instead of a complicated set of longitudinal tunnellike chambers; and several intermediate stages between these two extremes.

Preliminary to a discussion of the literature on the subject and to an attempt to trace the principal steps in the attainment of the present state of our knowledge of the anatomy of the calciferous glands, it is necessary to briefly summarize the principal facts of their structure, and explain the terminology used. In the Lumbricidae more commonly studied, the part of the esophagus which is included in somites 10-14, and is just anterior to the crop, is characterized by an extensive glandular development of the wall. This includes a pair of evaginated pouches in somite 10 which communicate through large apertures with the lumen of the esophagus. In the wall of the somites next following is a series of longitudinal tunnellike chambers which have their cavities continued into the posterior part of 10 where they are in communication with the large cavities of the paired pouches in that somite. Figure 17 illustrates the appearance of a transverse section, and figure 50 of a sagittal section through the wall of one side in this region. Longitudinal blood vessels extend along the margins of the lamellae which separate the longitudinal chambers, both along the inner margins in close relation to the lining epithelium of the esophagus and also along the outer margins in close relation to the muscle layers of the esophagus. These vessels provide an extensive blood supply and account for frequent references to the reddish color. The chambers are much reduced in size in somite 14 and the diameter of the esophagus is correspondingly reduced. In a very few species including the much described *Lumbricus terrestris*, the lateral walls of the esophagus are considerably expanded in each of the somites 11 and 12 and this has led to the common practice of referring to three pairs of glands in somites 10, 11 and 12, when in reality we are dealing with what would more reasonably be considered as a single organ. The pouches in somite 10 are the first or anterior pair of glands and the lateral expansions in somites 11 and 12 are the second and third pairs mentioned in much of the literature, including most zoology text-books.

HISTORICAL

The following discussion of the literature, as well as of the worms themselves, is limited to facts of the general anatomy or structure and deals not at all with the details of histology or of physiology. These latter topics are of great interest and importance, but have received little attention from the writer who therefore has no contribution to make in these fields. The literature examined includes most of the papers commonly mentioned by writers dealing with the subject and probably all, or nearly all, of those making important contributions, but no claim is made for a complete study, nor that some really important contribution may not have been overlooked.

Any who are interested in the histology and physiology of the glands are referred to a paper by Stephenson and Prashad (1919) for a discussion of the literature on those subjects.

As a matter of convenience in the following discussion, the usual practice of treating the gland region as made up of several pairs of glands, instead of a single one, will be followed.

The first paper to receive attention is that by Julius Leo (1820), entitled De structura lumbrici terrestris. Dissertatio inauguralis.

Approximately 30 pages of text and two plates are given to a general description of *Lumbricus terrestris* and of another lumbricid of smaller size. The writer was much surprised at the amount of accurate information contained in Leo's paper and still more surprised, in reading the papers of later writers, to discover how little was known by most of them about the contents of Leo's paper. Since the original article may not be easily accessible to some who might be interested in its contents, that part of it which deals especially with the calciferous glands, and the two figures of especial interest are here reproduced.

"Superficiem externam oesophagi, corpuscula oblonga et subrufa occupant, utrinque tria (vid. Fig. VII. ff. gg.).[1] Descriptionem eorum addam.

"In medio oesophago sita sunt et tanta vasorum multitudine abundant ut in verme dum vivit secto pulsantia videantur, unde etiam a Willisio cor animalis dicebantur. Bina anteriora e duobus sacculis membranaceis constructa sunt, et orificia eorum oblonga in oesophago discernere licet: (vid. Fig. VII. ff.) et (Fig. VIII. c.)[2] concrementi albi terreo-salini granula continent. Quae supersunt corpuscula quatuor formam cylindraceam habent, et e striis albis longitudinalibus transversim plicatis, constructa sunt. Orificia horum corpusculorum in oesophago nunquam vidi, cum sacculis autem antecedentibus coniuncta mihi videbantur.

"Qui sit corpusculorum usus me fugit, cum in omnibus animalibus analogiam invenire non successerit. Analysis chemica materiae terreo-salinae, qua corpuscula anteriora repleta sunt, naturam harum partium forsitan docebit. Huius substantiae excretionem magni momenti esse, eamque forsitan digestionem promovere ex eo verisimillimum est, quod corpuscula sanguinis copia alia organa superant."

It will be noticed that Leo recognized the first pair of bodies as being saccular and provided with definite openings into the esophagus which he represented in his figure VIII and which is reproduced as figure 2 in this paper. He also recognized that the second and third pairs were different from the first pair in structure, and had a better idea of what that structure was than have some others who have written long since his time. He also expressed a correct opinion concerning the communication between the last two pairs and the first pair, and of the absence of openings from the last two pairs directly into the esophagus. Although his figures are very simple, they are more accurate than many that have been contributed by later writers. He made no statement that exactly located the organs in definite somites.

An extended review of this paper together with a plate, on which were reproduced seven of the ten figures of the original paper, appeared with the same title in Isis (1822, pp. 492-496, pl. IV.). This review contained merely a brief reference to the calciferous glands, and reproduced the two figures illustrating them. "Weiter hinten liegen auf der Speiseröhre 3 Paar braune Körper (Fig. 7, ff. gg.) in denen sich kalkige Körnchen finden, welche auch in der Zeichnung nicht gehörig abgesondert sind und deren Nutzen nicht errathen ist. Die 2 vorderen sind haütige Säckchen and öffnen sich in die Speiseröhre. Willis hielt diese Körper, weil sie ganz mit Blutgefässen durchzogen sind, für das Herz."

Later writers who have seen only this abstract have necessarily incomplete information of Leo's contribution to our knowledge of the subject.

[1] A drawing from this figure is reproduced as figure 3.

[2] A drawing from this figure is reproduced as figure 2.

A brief paper by Leo entitled "Ueber die Fortpflanzung der Regenwürmer" appeared in Isis, 1820, p. 386; and a brief review of it in London Magazine, 1823, p. 556. Nothing pertaining to the calciferous glands is contained in this paper, or in the review article. They are mentioned here since references to them have appeared in literature lists included in papers dealing with the glands together with other topics.

C. F. A. Morren (1826) contributed an extensive paper which described the results of his observations on various phases of anatomy, physiology, and natural history of *Lumbricus terrestris* and allied forms. With certain additions and emendations this appeared later as a paper of XV+280 pages and 32 plates (1829) under a different title. A copy of the paper in this latter form is the one consulted by the writer. On page 129 of this paper under the caption De glandulis oesophageis, we have the following brief and imperfect description:

"Praeter glandulas quas exhibet membrana de quâ modo egimus, adhuc existunt quatuor corpora glandulosa, maxime distincta, quae circumdant oesophagum versus tertiam ejus partem posteriorem, ubi ovaria inseruntur. Illa corpora quae naturae glandulosae arbitror, per paria sunt disposita: unum in utroque latere, ita ut omnia uno puncto conjungantur in canale ipso oesophagi, quod speciem crucis efformat, (vide *i et k*, *fig.* 1 *et* 2. *tab.* X. *bis*). Sunt plus minusve eminentes; color earum variat ab albo ad subflavum rubescentem; tunica est dura, aequalis, laevigata, humectata, etc.

"De usu harum glandularum non constat; praesertim quum consideramus hyeme durante et in multis juniorihus Lumbricis, nulla fere reperiri earum vestigia et saepe eas omnino desiderari. An pertinent ad organa generationis; an evolvuntur cum his tantummodo? Hunc nodum hìc solvere non valeo."

Figures 4 and 5 are copied from the portions of Morren's figures which represen,t the glands. He seems not to have seen the paper of Leo. On page 28c in a list of authors who had written on the Lumbricidae, there is a referene: Leo, (Lond. mag. Nov. 1823, p. 536 [should be 556]). On page 189, Leo's name is mentioned with an appended footnote giving the title of his paper with date 1822 and the following comment "Quod opus consulere mihi non datum fuit." In view of Leo's contribution to our knowledge of the calciferous glands, that of Morren seems practically valueless. The number and form of glands is given incorrectly; nothing is given of the details of their structure, nor of the relations which their cavities have with each other or with the lumen of the esophagus.

F. G. J. Henle (1835) in a paper devoted principally to other objects gave attention (p. 581) to the earthworm calciferous glands and referred to Leo with whom he agreed in his general account of the number of the glands and their relations to each other and to the esophagus. He made no statement concerning the internal structure of the posterior two pairs, but commented on variations in their size due to variations in the amount of calcium salts contained.

E. Ray Lankester (1864 and 1865) published an article which appeared in three parts and dealt with the general anatomy of worms designated as *Lumbricus terrestris* and *L. agricola*. Part I on pages 263-268 contained the matter dealing with the alimentary tract and calciferous glands. It was accompanied by plate VII of which four figures were concerned with the glands. Lankester described three pairs of "oesophageal glands" of which the first pair were placed in the 11th somite (12 as enumerated by Lankester) and the other two pairs were placed in the next following somite. Of the first pair he says "They are firmly attached to the walls of the oesophagus, but do not appear to have any communication with its interior." He termed them pouches and stated that the wall is thin, and that they frequently contain crystalline bodies which are probably carbonate of lime. He stated that the second and third pairs differ from the first in form and appearance and that they are smaller, and their walls much thicker. They all have an "immensely vascular surface" due to numerous parallel longitudinal vessels and are often of "a bright red color." He described a very peculiar internal structure of all three pairs which is seen when a thin vertical section is made through them. His description and a figure indicate an interior "vascular region in which the blood vessels are arranged in loops."[3] No statement is made indicating that any communication exists between the cavities of any of the glands with each other or with the lumen of the esophagus. Lankester listed the review article of Leo's paper which appeared in Isis, 1822, but not the original article. Since he began his account by speaking of "three pairs of remarkable glands, which have never yet been described"; and mentioned Morren first in his account of the work that others had done on them, it seems reasonable to suppose that Leo's description had not been seen by him. The paper of Henle (1835) was not included in his bibliographic list nor mentioned in the discussion. Lankester's chief contribution was in his better figure of the superficial appearance of the glands and his description of the peripheral blood supply. His ideas of the relations of the gland cavities to each other and to the aesophageal lumen, and also of the structure of the second and third pairs of glands were less in conformity with facts than were those of Leo.

D'Udekem (1865) had a brief comment on the "glandes oesophagiennes." Both his brief description and a figure (7) show that he had an erroneous conception of their structure. His figure 2 shows more accurately the relative position of the glands and the associated lateral "hearts" than did a similar one of Lankester. He made no statements concerning their exact situation; nor concerning the relations of the cavities of the different glands to each other or to the esophageal lumen.

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[3] A drawing from this figure is reproduced as figure 7 and one from another one which has been copied in some modern text-books is reproduced as figure 6.

Claparède (1869) in a paper of about 60 pages and several plates published some important results of extensive histological studies of *Lumbricus terrestris*, and discussed in a thorough-going manner the published results of previous writers on the anatomy of Lumbricidae. By the use of more modern methods of technique and the study of transverse and longitudinal sections, he was enabled to add much information concerning the details of structure of the calciferous glands. His figures[4] and description of the transverse sections of the second and third pairs of glands and of the wall of the esophagus in the somites containing them furnish us the first successful attempt to make known the peculiar structure of that part of the alimentary tract. They established the presence of the longitudinal chambers or "follicles," and the separating lamellae which subdivide the space between the inner epithelium and the outer muscle layers of the esophageal wall. They also made known the presence of the series of longitudinal blood vessels which are distributed between the inner boundaries of the longitudinal chambers and the epithelial layer of the wall. The existence in the middle of each lamella of vascular connections between the outer and inner series of longitudinal blood vessels was also made known. The fact that what were termed second and third pairs of glands were merely lateral expansions of the glandular wall with which they were related was made clear for the first time.

Claparède believed that the anterior pair of glands was in somite 11, and that the so-called "second pair of glands" was close to them in the posterior part of the same somite, and that the third pair was in the 12th somite. Not only did he fail to correctly locate the glands, but he also failed to recognize the direct communication of the cavities of the three pairs of glands. He seems to have believed that the contents of the "follicles" or chambers of the posterior glands discharged their contents through small clefts of the epithelium into the lumen of the esophagus; and in a footnote, although he intimated the correctness of Leo's claim that there were no large openings for such passage, he claimed that Leo's opinion that the cavities of the three pairs were united was incorrect. "Diese Abwesenheit von grösseren Mündungen an den Blätterdrüsen war bereits dem Leo[5] aufgefallen. 'Orificia horum corpusculorum in oesophago nunquam vidi' sagt er. Darauf setzt er hinzu: 'cum sacculis autem antecedentibus conjuncta mihi videbantur' (loc. cit. p. 14), was entschieden unrichtig ist." Two factors presumably contributed to his failure to find the passage-ways between the cavities of the second pair of glands and those of the first pair. The narrowed connecting passages are often somewhat tortuous, (depending perhaps on the degree of contraction of that region of the worm when killed), and are correspondingly difficult to follow in sections. Serial sections are almost essential for tracing them in many specimens, but the technique involved in the preparation of such sections was not developed until a considerably later date (1881). Claparède's failure to understand the structure and function of the first pair of glands is indicated by the following footnote on page 606: "Unmöglich ist es auch nicht, dass der Stoff zu den grossen Kalkconcrementen der vorderen Säcke ursprünglich von diesen Drüsen abstammt und durch eine Art Regurgitation bis in diese Säcke geleitet wird. Der Umstand, dass die Wand dieser Organe keine drüsige Structur, sondern blos die normalen Schichten, selbst flimmenndes Epithel bietet, würde zu Gunsten dieser Ansicht sprechen."

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[4] Drawings from two of these figures have been reproduced as figures 8 and 9.

[5] In a footnote on page 617, Claparède discussed the failure of various investigators to recognize the important contribution of Leo to a correct understanding of the function and relations of the spermducts, and referred to a figure which was included in Leo's paper This figure has been copied and reproduced as figure 1 in the present paper, since it was the occasion of a very apt comment of Claparède which applies to Leo's noteworthy success in his study of the calciferous gland as well as of the spermducts. "Das Verdienst der ersten Entdeckung und genauen Erkenntniss gebührt nichtsdestoweniger dem jetzt ziemlich vergessenen Leo."

Perrier (1874) did not definitely state that the three pairs of "glandes du calcaire" are in the 10th, 11th, and 12th somites; but, he stated that they are in the same somites as the "testicules" (p. 351) and elsewhere (p. 367) located the testicules in somites 10, 11, and 12, therefore it seems probable that he was aware of the actual position. This is the earliest account known to the writer in which they are correctly located. He claimed that all three pairs of glands have fundamentally the same type of glandular structure, all having flattened glandular lamellae. In other respects, his anatomical account added nothing to that of Claparède, and was less accurate when he stated: "Ces feuillets sont placés entre la couche vasculaire et les couches musculeuses de la paroi oesophagienne." The importance of Leo's observations were apparently less obvious to Perrier than they were to Claparède.

Horst (1876) in a paper of about 30 pages with one double plate has given the results of his studies of the anatomy of *Lumbricus terrestris*. He described three pairs of calciferous glands, located in the 10th, 11th, and 12th somites. A figure which shows the relations of the principal blood vessels of the first dozen somites makes it obvious that Horst had a definite and correct conception of the location of the three pairs of glands and of the lateral "hearts" associated with them. He was no more satisfactory than Claparède in his discussion of the relations of the cavities of glands of one pair to those of others and to the lumen of the esophagus. He wrote of his inability to find the clefts in the epithelium through which Claparède supposed there was a communication of the cavities of the second and third pairs of glands with the esophageal lumen. Frequent references are made to the papers of Lankester, Claparède and Perrier, in his discussions of both the anatomy and physiology of the glands.

Darwin (1881) devoted several pages to a discussion of the functions of the calciferous glands, but accepted Claparède's ideas concerning the relations of the glands to each other and to the esophageal lumen. The belief that there is no direct communication between the cavities of the first pair of glands and the chambers or "follicles" of the second and third pairs led to his misinterpretation of the narrow connecting passages affording such direct communication.

He commented on the slight amount of information contributed by Claparède concerning the structure of the first pair of glands, and on his assumption that the calcareous matter forming the concretions is derived from the other two pairs of glands. Darwin mentioned the presence of lamellae in the first pair of glands when they contain only small concretions and made the following statement. "After the formation and expulsion of a large concretion, new lamellae must be developed in some manner. In one section made by my son, the process had apparently commenced, although the gland contained two rather large concretions, for near the walls several cylindrical and oval pipes were intersected, which were lined with cellular matter and were quite filled with free calciferous cells. A great enlargement in one direction of several oval pipes would give rise to the lamellae." It seems to the writer altogether probable that these "pipes" were the narrowed connecting passages between the cavities of the first and second pairs of glands. As a result of a number of observations and considerable thought Darwin reached the conclusion: "With respect to the function of the calciferous glands, it is probable that they primarily serve as organs of excretion, and secondarily as an aid to digestion."

Vejdovský (1884) made but brief references to the calciferous glands of Lumbricidae and announced his intention to discuss them at greater length in a later publication in which not only the glands in Lumbricus but those in other locally distributed genera would receive attention. He gave the location of the glands in 10, 11, and 12.

Marshall and Hurst included the earthworm among the forms described in their Practical Zoology of which the first edition appeared in 1887. The writer has not seen a copy of the first edition, but, on the assumption that the principal features of the description of the calciferous glands agreed with those in later editions, they seem to have been the first workers since Henle (1835) to confirm some of the more important observations of Leo (1820). They described the second and third pairs of glands as communicating with the first pair which open directly into the lumen of the esophagus. The description of the structure of the second and third pairs of glands agrees in the main with that of Claparède (1869). They located the first pair of glands in 10, as had Perrier (1874), Horst (1876) and Vejdovský (1884).

Kulagin (1888) contributed brief notes on several topics connected with earthworm anatomy, some of which dealt with the calciferous glands. He referred especially to *Lumbricus rubellus*, "*Allolobophora mucosa*" (=*Helodrilus roseus*),[6] and *Allolobophora* (=*Helodrilus*) *foetida*. He mentioned the first pair as "Falte des Oesophagus von der rechten und linken Seite," divided internally, and with openings into the oesophagus always distinct. He referred to the communication between glands of the second and third pairs where they are in contact as something not previously announced. He wrote of the second and third pairs of glands of *H. roseus* as not lying on the right and left sides of the esophagus, but, as surrounding it. This is the earliest definite statement noticed by the writer concerning a type of calciferous gland anatomy which in more recent times has been found to be of very frequent occurrence.

Rosa contributed quite a large number of papers in which are found descriptions of various species of Lumbricidae. Brief statements concerning the calciferous glands are found in a considerable number of these descriptions. The writer has not noticed any detailed account, in any of Rosa's papers, of the internal structure or relations of these glands, but statements concerning the number of pairs of glands, their superficial appearance, and the location of the somite or somites containing them occur frequently. In at least two of them (1887 and 1893, p. 15) attention was called to differences in the number of glands present in different species; three pairs in *Lumbricus herculeus*, in 10, 11, and 12; but one pair, in 10, in a considerable number of species. In one paper (1895), he described a species having one pair of glands, in 9; another with but one pair of glands, in 10; another with but one pair of glands, in 11; while in still another one (*Allolobophora robusta*) they are not superficially recognizable. Rosa in his recognition of the number and location of the calciferous glands as useful characters in distinguishing species of Lumbricidae was later followed by Cognetti.

Beddard (1895) gave an extended account of the various types of calciferous glands found in the different families of earthworms. Those of Lumbricidae were discussed but briefly. Claparède is mentioned as having studied the minute structure of the glands in Lumbricus. Marshall and Hurst were given credit for being the first to correctly locate the first pair of glands in 10, and also the first to correctly describe the relation between the cavities of the three pairs of glands with each other and with the lumen of the esophagus. As has already been stated, credit for the first announcement of these facts belongs to others.

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[6] The genus name Helodrilus Hoffmeister was adopted by Michaelsen (1900a) as the technically correct name for a group of Lumbricidae which had for a considerable number of years been known under the genus name Allolobophora Eisen. The change in name was assumed to be necessary for strict conformity with requirements of certain accepted rules of nomenclature. Most systematists in this group followed his example. In recent years Michaelsen and some others have returned to the use of the name Allolobophora. The writer knows of no authoritative decision that Helodrilus is not the technically correct name and hence it is used in this paper.

Moore (1895), in the description of "*Bimastos palustris*," an interesting species of Lumbricidae from the vicinity of Philadelphia, Pennsylvania, compared the structure of the calciferous glands with those of *Lumbricus terrestris*, as described by Claparède. He found that the modified part of the esophageal wall with its tunnellike chambers and separating lamellae extended to the 14th somite, although the width of the lamellae and chambers was considerably less in this region. This is the earliest account noticed by the writer in which the posterior end of the calciferous gland structure has been correctly located. A further discussion of Moore's paper will be found on a subsequent page in connection with a statement of the results of a study of specimens of this species by the writer.

Harrington (1899) gave the most complete and in some respects the most accurate account of the Lumbricid type of calciferous gland that appeared in the 19th century. His studies were based on the glands of *Lumbricus terrestris* and his treatment of the anatomy and histology was but a foundation for his presentation of the results of his studies of the physiology of the calciferous glands. The author died without having revised the proofs of his paper and some errors, to be discussed later, might otherwise have been eliminated. He listed the paper of Leo and made two brief references to his work, but apparently was not aware of the more important discoveries announced by that investigator. "Since Julius Leo, in 1820, discovered lime-secreting organs leading into the digestive tract of earthworms, very many workers have examined and reported observations upon these interesting structures." "Julius Leo ('20), who first described the calciferous glands, says concerning them: 'Qui usus corpusculorum fugit me.' " This quotation from Leo is also contained in a footnote in the paper of Claparède. Harrington credited to Morren the suggestion that the glands might furnish lime for the egg capsule. This suggestion was made by Lankester (1894, p. 266). Lankester was credited with observing "that the calciferous glands opened into the alimentary tract," although Lankester stated that he found no such openings. Mention is made of some of these errors because they seem to account for errors in certain statements of a more recent writer. Certain errors in the list of literature will receive later attention.

Harrington announced two discoveries that involved important changes in the ideas of the structure of the glands. "The cavities are also continuous from somite X to XIV, opening at the former into the hollows of the first pair of glands, and at the latter directly into the oesophagus." (p. 108-109). The same idea is advanced in another place (p. 111-112). "If it (lime) is passed backward, it breaks through oesophageal epithelium, in this case opening directly into the digestive tract at the middle of XIV. This second outlet for the secreted product has escaped the notice of previous writers." This assertion of the presence of normal posterior openings for the gland is adopted by one later writer (Combault, 1909) and disputed by Stephenson and Prashad (1919). The present writer is not convinced that such openings are normally present.

The second announced discovery mentioned above is outlined as follows: "The anterior pair of pouch-like glands are the only ones lined with true epithelium and are developed as diverticula of the oesophagus, while the two posterior pair are formed by migration of the amoeboid endodermal nuclei, which arrange themselves about cavities formed *de novo* near the splanchnopleure and give rise to the secretory elements." (p. 137).

"The unusual relations between the glandular and circulatory systems can be interpreted only by regarding the one-layered secretory lamellae bounding the blood spaces as greatly hypertrophied vascular walls representing both the intima and endothelium." (p. 106). This idea also is adopted in part by Combault and is repudiated by Stephenson and Prashad. In view of some recently ascertained facts to be discussed subsequently in this paper the writer believes that it is erroneous.

The major part of the paper is devoted to discussions and descriptions of the histology, physiology, and blood supply of the glands, which are subjects not included in the scope of this paper.

Ribaucourt (1901) published a paper of about 100 pages dealing with the comparative anatomy of the Lumbricidae and based especially on European representatives which were studied by modern histological methods. The "Glandes de Morren" received attention to the extent of about ten pages and some sixteen figures, based on studies of the glands of nine or ten different species representing several genera. The work had been carried on simultaneously with that of Harrington, but apparently neither knew of the work of the other. Ribaucourt called attention to the tendency of previous writers to assume that the glands of Lumbricus, which were the ones most studied, were representative of those belonging to worms of other genera, "ce qui est une erreur." No attempt was made to compare the results of his studies on the glands with those announced by previous workers. He simply described the glands of Lumbricus as he saw them, and then the glands of nine other species were compared with them, with especial attention to differences. Diagrammatic figures of longitudinal frontal sections of the glands of nine species were given, and these include representatives of six different genera of Lumbricidae which he recognized. Some of these genera are usually treated by systematists of the present time as subgenera.

Ribaucourt made no reference to the precise location of the glands and but little is stated about the relations at the posterior end. Some of his plate figures indicate that the anterior end is in 10. In his discussion of the glands in Lumbricus, he went one step farther than Claparède or Harrington by his treatment of the series of longitudinal chambers in the wall of the esophagus, extending between the anterior pair of glands and the crop, as a distinct unpaired gland which he called "la glande antéro-postérieure," and designated as IV. He considered this to be the more primitive member of the series of glands and that the three pairs commonly recognized were secondary differentiations from it. "La glande antéro-postérieure de Morren est la plus ancienne, l'antérieure et les deux moyennes II, III n'en sont que des différenciations secondaires." As a result of his comparative studies of the glands of various species representing different genera of Lumbricidae, he found that the most simple form of gland development in the forms examined was that of *Helodrilus hermanni* in which only the gland IV was present. There were no paired anterior pouches nor paired expansions in next following somites. (This species is considered by Michaelsen (1900a), who originally described it, to be a synonym of *H. oculatus*.) Between the type of calciferous gland development in this species and that of *Lumbricus terrestris* occurred various intermediate degrees of complexity. Absence of the lateral expansions, designated by Ribaucourt as II and III, was especially common.

Combault published several brief papers in 1907, in which various topics related to the structure and physiology of the "glandes calcifères des Lombrics" were discussed. Since a more extended article by him (1909) presented in a more complete and unified form the results of his studies of these organs, attention will be given only to the contents of that paper. "Contribution a l'étude de la respiration et de la circulation des Lombriciens" is the title, and was the natural outcome of the author's opinion that the "glandes de Morren," as the glands are called in this paper, function chiefly as organs of respiration. The third division of the paper (pp. 24-35) entitled "Anatomie des glandes de Morren" contained the matter which will receive chief attention here. His historical account contained allusions to the earlier writers, which were obviously based on Harrington's statements and repeated the errors made by that writer. Leo is mentioned as the first to call attention to calciferous glands. His inability to ascertain their function was announced, but there was no mention of his important discoveries pertaining to the relations of the glands to each other and to the esophagus. Credit for these discoveries was given to later writers.

Combault gave Lankester credit for first describing the openings of the anterior pair of glands into the esophagus, and Claparède was mentioned as confirming that result. Leo first described and figured such openings. Lankester expressly stated that he did not find them, and Claparède quoted the statements of Leo in which they are mentioned. It is obvious that the paper of Claparède received little or no attention from Combault, since he made a statement implying that Lankester alone, of the various investigators who published before Harrington and Ribaucourt, used modern methods. All others depended on dissections. Such a statement would be highly improbable from any one who had seen Claparède's detailed account of the various methods of fixation, staining, clearing, and mounting of sections; and who had examined the half dozen plates which illustrate the paper, and of which the majority of the figures were based on sections.

Combault described and figured the glands as located in somites 11 to 14, instead of having the anterior part or pair in 10. When the lateral "hearts" were figured they were represented in the correct relation to the glands since the posterior two pairs of hearts were shown associated with the anterior two pairs of glands. He agreed with Harrington in claiming that there are paired openings into the esophagus at each end of the gland region, even though admitting that such openings were not recognizable in 14. About the only real contribution or advance in our ideas of the anatomical relations of the calciferous gland structures, made by Combault, is the suggestion that the glandular region in the posterior part of the esophagus in Lumbricidae should be considered as one gland instead of several pairs of glands. The major part of the paper is given to discussions of the histological and physiological features of the glands and has been reviewed by Stephenson and Prashad (1919). The bibliography contained nearly 200 titles, some of which included errors that had occurred in the lists of previous writers and presumably were incorporated without checking for determination of correctness. Harrington listed a paper: '42 Henle, F. G. J. Müllers Archiv. pp. 238-280, 1842. The paper was really by Dr. Friedrich Stein. Precisely the same reference occurs in the list of Combault. A typographic error in the reference to the paper of Perrier (1874) in the literature list of Vejdovský (1884), which indicated the pages as 331-350 instead of 331-530, was reproduced in Harrington's list and again in that of Combault.

In a paper which was published in 1917, the writer included in the brief summary of distinguishing characters of each of about a dozen species of Lumbricidae the approximate number of the lamellae separating the gland chambers. The expression "longitudinal partitions" was used and reference was made to "the calciferous gland." The number of the partitions was necessarily approximate, since there is some variation in the number in different parts of the same organ as well as individual variation. These numbers are perhaps not very significant except where there is a considerable divergence. It seems improbable that such numbers as 40 and 60 would be found in different adult individuals of the same species unless represented by different varieties.

Stephenson and Prashad (1919) contributed an article on the calciferous glands of earthworms which dealt with these organs in representatives of three different subfamilies of Megascolecidae and of two genera of Lumbricidae. The discussion of the last named family included descriptions of the structure of the glands in young and adult *Helodrilus caliginosus;* in *H. parvus*; and in a species of Lumbricus, presumably *L. terrestris.* The morphology of the glands, including the histology and general anatomy, received chief attention; and there was an extended review of papers of Harrington and Combault in which their peculiar views and statements concerning the histology and physiology of the glands were carefully discussed. The authors presented evidence and arguments in support of their contention that the complex type of gland found in the Lumbricidae has had its origin in a more simple type of gland. "The condition in the Lumbricidae originated in a series of longitudinal lamellae. The mode of evolution has been comparable to what has happened in *Eutyphaeus*—the inner edges of the lamellae have fused. In this way a series of longitudinal tunnels has been formed, in and part of the epithelial coat of the oesophagus, and entirely within the muscular coat. These tunnels open in front, where the longitudinal folds begin, into the oesophageal pouches in segment X; they become progressively smaller, and cease in XIV without posterior openings.

"The epithelium of the glands is in all cases continuous with that of the oesophagus, and comparative anatomy shows that the various forms of glands are essentially due to various forms and degrees of complexity of the epithelial folds. The glands are therefore not mesodermal in origin, and are not merely the walls of blood-vessels, as has recently been contended."

They agreed with the suggestion of Combault that the gland should be considered as a single organ rather than as several pairs of glands. In the historical résumé of the contributions of earlier works on the calciferous glands, they began with that of Lankester who was credited with being "the first to examine them in any detail, though Morren had given a rough figure without any accurate description." The papers of Leo (1820) and Henle (1835) were not mentioned. Some of the better known text-books received attention and Marshall and Hurst were credited with being the first to correctly give the location of the glands. Their description was outlined without adverse comment other than that implied in the general statement applying to all of the text-books mentioned: "We do not think that a student would arrive at an adequate conception of the glands from any of these descriptions."

An examination of about 45 different high school and college text-books in which the calciferous glands are discussed and which are in use in the United States, or have been within recent years, has made it very evident that in the majority of cases the writers have relied chiefly on the statements and illustrations found in previously existing text-books, as a basis for their own statements and descriptions. Certainly the majority of the authors could not have examined with any care a series of sections through that part of the esophagus located in the somites 10-14, or have very closely studied a careful dissection of that part of the esophagus in *Lumbricus terrestris*, which most of them obviously attempt to describe. The least faulty descriptions are usually those of which the ancestry can be traced back to the Marshall and Hurst text-book. But one of the authors (Hegner) gives evidence of familiarity with the paper of Harrington, which appeared in a well known and much used American journal. There may be ample justification for omitting any account of the calciferous glands from our text-books, but there seems to be no valid excuse for statements that are contrary to facts. Statements like one which appeared in a recently published American text-book are more than a half century behind the times, since they have less that is true and more that is untrue than is found in the combined accounts of the calciferous glands, by Claparède in 1869 and Leo in 1820. The statement to which reference is made is as follows: "The oesophagus begins at the posterior end of the pharynx in the region of the sixth segment and continues posteriorly as a thin-walled, undifferentiated tube to the fourteenth segment, where it connects with the crop and gizzard. Three pairs of calciferous glands open into the oesophagus near its posterior end." The part of the esophagus which is in somites 10-14 is not a thin-walled, undifferentiated tube. The calciferous gland development is in the esophageal wall and included between the epithelial layer, which surrounds the lumen, and the muscular layers which form a part of the wall. In the absence of any statement to the contrary, the natural implication would be that the glands of the three pairs open in a similar manner into the esophagus, which is not true. The esophagus certainly does not connect with the gizzard at the "fourteenth segment."

The text-book description criticized above is by no means unique. It illustrates a kind of treatment of the subject that is to be found in a considerable number of those in common use. Most of the authors have exhibited a tendency to depend too much on statements found in other text-books.

A brief résumé of the various discoveries leading up to our present day knowledge of the calciferous glands of the Lumbricidae may be stated, in approximate chronological order, in the following manner: In *Lumbricus terrestris*, the posterior part of the esophagus has three pairs of glands. Those of the anterior pair are saclike in nature and their cavities communicate with the lumen of the esophagus through paired oblong openings in the lateral walls of the esophagus, one such opening for each of the two glands.

The other two pairs form a sort of cylinder containing longitudinal folds or lamellae which are radially arranged. No openings from these two pairs of glands leading directly into the esophageal lumen were found, but they seemed to communicate with the anterior pair. Leo in 1820 gave us this much of a start. Lankester in 1864 showed that there is a conspicuous series of parallel longitudinal blood vessels related to the outer surface of these glands. Claparède in 1869 made known a similar series of parallel longitudinal blood vessels related to the inner layer of the gland formation. He also furnished a much more definite idea of the nature of the longitudinal lamellae which separated the longitudinal chambers or follicles in the second and third pairs of glands, and showed that the middle or vascular layer of the lamellae furnished vascular communications between the two series of longitudinal blood vessels already mentioned. He made clear for the first time that what are termed the second and third pairs of glands are lateral expansions of the glandular esophageal wall with which they are related.

Perrier in 1874 announced that the first pair of glands contained flattened glandular lamellae like those of the other two pairs. He did not definitely state which somites contained the glands but from what he did state we are led to infer that they are in somites 10, 11, and 12. This was the first account furnishing data for their correct location. Horst in 1876 definitely located the glands in 10, 11, and 12. Statements and figures alike give that location. Marshall and Hurst in 1887 in their Practical Zoology confirm the opinion of Leo, that the cavities of the second and third pairs of glands communicate with those of the first pair and through them with the esophageal lumen. It took two thirds of a century to get this opinion of Leo definitely established.

Moore in 1895 definitely determined the posterior end of the calciferous glandular formation in one species of Lumbricidae in the 14th somite; but the earliest definite statement correctly locating the posterior end of the gland in *Lumbricus terrestris*, that has been noticed by the writer, is that of Harrington in 1899 who also found it to be in somite 14 in that species.

Combault in 1909 made the suggestion that instead of three pairs of glands, it is more reasonable to consider that we are really dealing with a single calciferous gland.

Stephenson and Prashad in 1919 presented arguments in support of a claim that the calciferous gland has had its origin in a glandular development involving the formation of a series of longitudinal folds of the lining epithelium of which the inner or free margins have fused, giving rise to a series of tunnellike chambers included between the muscular layer of the esophageal wall, and the epithelial layer, which surrounds the lumen of the esophagus.

An interesting parallel to such a supposed series of intermediate stages culminating in the complex organ present in modern Lumbricidae is found in the calciferous glands of various species of Diplocardia. They are described in another part of this article.

Rosa, first in 1887, and subsequently in other papers, mentioned several species in which there is but one pair of calciferous glands, those being located in the tenth somite. Kulagin in 1888 stated that in one species the second and third pairs were not laterally placed but surrounded the esophagus.

Ribaucourt in 1901 described the calciferous glands in a considerable number of different lumbricid species showing much variation in the appearance of the glands. One of them was assumed to be of an ancestral type, since it showed no lateral enlargements of the glandular wall.

TYPES OF CALCIFEROUS GLANDS IN LUMBRICIDAE

The more important general facts of the anatomy and of the relations of the cavities in the different parts of the gland to each other and to the esophageal lumen have already received attention. We have now to consider some of the various types of glands found in different species of Lumbricidae. As has already been pointed out by different writers, the essential feature of the gland is the series of longitudinal chambers contained in the esophageal wall of several somites and ordinarily terminating anteriorly in 10 and posteriorly in 14. These chambers and the lamellae by which they are bounded occupy the greater part of the space produced by the separation of the inner epithelial layer of the esophageal wall from the muscle layers in the outer part of the wall. The so-called vascular layer normally included between the epithelial and muscle layers of the esophageal wall is very intimately associated with the walls of the gland chambers being represented in the longitudinal blood vessels extending along the outer and inner margins of the chambers, and in the vascular middle layer of the separating lamellae. These chambers are continuous throughout the length of the gland; they apparently end blindly in 14 without normal openings into the esophageal lumen; and they usually open anteriorly in 10 into cavities continuous with the lumen. The anterior openings may be into cavities of relatively large evaginated pouches occupying a great deal of space in 10, or the cavities may be insignificant and but slightly differentiated from the lumen. In two species, at least, the anterior openings are in 11, instead of in 10. There may or may not be lateral expansions of the gland in one or both of somites 11 and 12.

The ease or difficulty of a study of the glands is dependent on several different factors. Important among these is the state of contraction existing in the anterior part of the specimen at the time of fixation. The state of preservation is important, especially when one is attempting to determine the presence or absence of posterior openings of the gland chambers. The general plan of structure is an important factor in an attempt to demonstrate the continuity of the chamber cavities in the anterior part of the gland with cavities that are connected with the esophageal lumen. In specimens of some species in which the outer part of the gland wall is nearly cylindrical in the two or three anterior somites and without noticeable constrictions where the septa are attached, such continuity may be obvious in a single sagittal section (Fig. 50). In other specimens with a type of gland in which the diameter of the gland at 10/11 is much less than in the part lying anterior to the septum, an examination of a considerable number of consecutive sections is sometimes necessary before such continuity can be determined.

Figures of longitudinal sections of the glands have been prepared in most cases from sections cut in the frontal plane, since the esophageal pouches and characteristic enlargements of the gland in some species are chiefly lateral in position and hence are shown to the best advantage in such sections. Because of the unavoidable irregularities in the position of the various parts of such a long organ of complicated structure, in specimens often more or less contracted and contorted, the frontal sections that show the characters to the best advantage for one part of the organ are almost certain to be unsatisfactory for some of the other parts. Sections necessarily pass obliquely through the wall chambers and lamellae in some parts of the organ and often in most parts. Diagrammatic figures like those of Ribaucourt and Combault are so unsatisfactory that it was decided to use for this paper camera drawings from sections, in the belief that they would be at least as useful and more accurate. Not infrequently the histological condition of the only specimens available for sectioning has been unsatisfactory.

ESOPHAGEAL POUCHES WANTING

The species first considered are those in which there is an absence of paired lateral evaginations of the esophageal wall in somite 10. They lack the paired esophageal pouches, called by Ribaucourt and Combault "diverticules de Perrier," and often designated as the "first pair of glands." The simplest form of glands of this type in the Lumbricidae known to the writer is found in *Helodrilus oculatus*, a European species, and will be the first to receive attention. The glands of three other species included in this group have been studied and will be described. These species are ***Helodrilus venetus*** and the variety ***H. v. hortensis***, ***H. foetidus***, and ***H. lönnbergi***.

Helodrilus oculatus Hoffmeister. Ribaucourt (1901), as previously noted (page 18), gave a brief description of the gland in this species, using a former name, *Allolobophora hermanni*. The writer is fortunate in having two specimens which were collected in Europe, and identified and presented to him by Dr. Michaelsen, some years ago. Transverse sections of the anterior end of one of these specimens make possible certain additions to the description by Ribaucourt. This is desirable since that writer assumed that the gland is of a more primitive type than are those of the other species examined by him. The anterior end of the gland is in somite 10, approximately midway between 9/10 and 10/11, and the posterior end is in somite 14. There is but little variation in the diameter of the esophagus or of its lumen in the different parts of the gland region, and in the esophageal somites next anterior to it. There are no lateral evaginations in 10 at the anterior end of the gland comparable to the "esophageal pouches" found in most Lumbricidae. Unpaired irregularities like those shown in Ribaucourt's figure (1901, Fig. 23) are not very obvious and when present may perhaps be temporary and due to a contraction of that part of the body of the worm.

The inner epithelial layer forms numerous short elevations or papillae in the anterior part of 10 (Fig. 11) and in the preceding somites, but in the gland forms a few low longitudinal ridges and shallow grooves. The character of the epithelium differs somewhat in the gland region where the surface has numerous short, fine, parallel processes projecting into the lumen. It is the type of epithelium described by Stephenson and Prashad (1919) and by them called rodlet epithelium.

As one follows a series of sections posteriad, beginning at the anterior end of the gland, the longitudinal chambers of the lateral walls are the first to appear (fig. 10), soon followed by those of the dorsal and ventral sides. The number of the chambers and their relative magnitudes, as seen in transverse section, are shown in figures 12 and 13. The openings of the chambers into the lumen at the anterior end of the gland are traced with difficulty and none are conspicuous. There is no appreciable change in the dimensions of the chambers where the septa connect with the esophagus, but there is a gradual decrease in the width of the chambers between the lamellae and a relative increase in the thickness of the lamellae, as one follows the series of sections posteriad (Fig. 14). This change in thickness is due, at least in part, to an increased amount of blood in the median vascular layer of the lamellae. A careful search has not led to the discovery of any communication between the posterior ends of the longitudinal chambers and the lumen of the esophagus. This was the simplest type of gland described by Ribaucourt and found by him in but one species. Rosa (1895), in his description of *Helodrilus robustus*, writes of the calciferous glands in a way that seems to indicate a similar type of gland in that species. "Le ghiandole di Morren non sono esternamente riconoscibili."

Helodrilus venetus var. *hortensis* (Michaelsen) is a widely distributed earthworm type, well known in Europe, and recorded from Asia, Africa, and both American continents. The only record from the United States, noticed by the writer, is by Michaelsen (1900) and was based on specimens from California. During the past two years more than three hundred specimens which are assumed to belong to this variety have been collected from the banks of a stream flowing through Urbana, Illinois. Serial sections of anterior parts of ten specimens have been examined as a basis for the statements concerning the calciferous gland of this form. Two of the series are sagittal; four, transverse; and four are frontal.

The calciferous gland of this form resembles that of *H. oculatus*, in the absence of evaginated pouches in 10, and in lacking any definite inflations in any one or more of the following somites. It more nearly resembles the glands of most other species in the general proportions of the chambers as shown in transverse sections (Fig. 17). The position of the anterior end of the gland is somewhat variable, but is in somite 11 in all of the specimens studied. In most of the specimens it is very near the anterior end of the somite, being but slightly posterior to the place of union of the septum 10/11 with the wall of the esophagus. In one specimen it is about midway of the somite and in another one is about one-third of the length of the somite from the anterior margin. These statements are made with the assumption that the part of the esophagus included between the septa 10/11 and 11/12 belongs to somite 11.

In *H. v. hortensis* the anterior ends of the lamellae have their inner margins free for a very short distance and two or more of them may be included in a single subepithelial cavity (Fig. 16) which opens through a single channel into the esophageal lumen. Although there are approximately 40 of the wall chambers, there may be less than half of that number of the channels opening into the lumen. The course of these channels may be at right angles to the long axes of the related chambers or may even be directed somewhat posteriad as they approach the lumen (Fig. 15). 38, 40, 40, and 43 are the numbers of wall chambers present in the glands of the four specimens of which transverse sections were made, as shown in sections near 11/12 in each. The width of the chambers in the plane parallel to the separating lamellae is somewhat greater in the anterior two or three somites of the gland than in the posterior ones, but the difference is not very marked. No communication of the cavities of the chambers at their posterior ends with the lumen of the esophagus has been found.

Helodrilus venetus (Rosa) in its typical form is well known in Europe and Asia. Three European specimens collected at Trieste were identified by Dr. W. Michaelsen and kindly presented to the writer a few years ago. Two of these have been sectioned in part and have given an opportunity for a comparison of the characters of the gland found in *H. v. hortensis* with that of the typical form of the species. The chief difference noted is in the number of wall chambers (Fig. 19), which is 61 in the single specimen of *H. venetus* typ. of which transverse sections were made, instead of approximately 40, as in *H. v. hortensis*. The anterior end of the gland in *H. venetus* is approximately midway of somite 10, instead of being in 11, as in the variety *hortensis*. The specimens of *H. venetus* were very strongly contracted and the glands correspondingly shortened and somewhat contorted. This fact probably accounts for the apparent inflations of the gland wall between the septa of its anterior somites. In the structural characters of the glands of the two forms, there is a marked similarity (Fig. 18).

Helodrilus foetidus (Savigny) is a very abundant and widely distributed species and has a gland that is similar in some respects to that of *H. v. hor-*

tensis. The anterior end of the gland is in somite 11 instead of 10 and is without paired evaginated pouches. Figures 20 and 23 show the chambers to be relatively narrow in 11; in 12 they are very much wider in the lateral walls (Fig. 20 and 24); and posterior to 12 they are again narrow, as in other species. Ribaucourt's description and figure 28 represent the general form correctly and his figure 63 shows the anterior end of the gland associated with the posterior pair of hearts indicating the 11th somite. Combault's figure represents the anterior end as relatively much larger than it really is, and his description places it in the same somite as he gives for the anterior end of the gland in other species, viz., in somite 11. This position happens to be correct for *H. foetidus* but incorrect for most others.

A study of transverse sections shows that the cavities of two or more chambers may be in communication with the esophageal lumen through one connecting channel (Fig. 21 and 22), as in *H. venetus hortensis*, but there is no corresponding display of free inner edges of lamellae grouped in subepithelial cavities like those found in that form (Fig. 16). The number of wall chambers is approximately 60.

Helodrilus lönnbergi (Michaelsen) was described in 1894 from specimens collected at Savannah, Georgia, and was later recorded from North Carolina (Michaelsen, 1900). The first specimens seen by the writer were immature individuals which were sent from the U. S. National Museum for identification. They had been collected by Dr. E. T. Wherry in an acid bog near Riverdale, Maryland, while he was carrying on certain investigations on acid soils. No clitellum was present and positive identification could not be made; hence an appeal was made to Dr. Wherry for more specimens to be obtained in the Spring. He accordingly collected additional specimens on March 9, 1924 and shipped them in living condition. Some of them were at the height of sexual activity and furnish the basis for much of the following description of the calciferous glands. Michaelsen had noticed the conspicuous enlargements of the esophagus in somites 11 and 12 and that they were of the nature of calciferous glands.

Transverse and longitudinal sections have shown that the gland of this species is fundamentally like those of other Lumbricidae, but it has superficial differences in general form that are conspicuous. The lateral enlargements of the esophagus in 11 and 12 in immature specimens occupy the greater part of the space in those somites and are due to the great width of the chambers and their separating lamellae (Fig. 82). The glandular development extends into 10, but the chambers in that somite are relatively narrow and there are no evaginations of the wall to form "pouches." The openings of the chambers into the lumen are very obvious and are illustrated in figures 25 and 27. In the latter (Fig. 27) which is slightly oblique, the right hand part of the figure represents a part of the section just at the anterior end of the chambers, while the left hand part of the figure is from a part of the section which is a little posterior to the chamber ends. On the right, the ends of the cavities of two chambers are shown communicating through a single short channel with the lumen. A similar relation is shown for the two chambers next above, but not quite so clearly, because the posterior wall of that short channel is included in the section. Other such communications are shown in other sections, and in one instance the ends of four chambers open through the same short channel. In somites 13 and 14 (Fig. 26) the gland is of small diameter and the chambers are relatively narrow. No evidence of any posterior communication of the chambers with the esophageal lumen has been found after careful search.

The gland contained much calcareous material in some sectioned specimens and similar contents were conspicuous in others that were dissected. A peculiar histological condition of the separating lamellae is obvious in most of the sectioned specimens and an attempt has been made to represent this in figure 29 which is from a section through the anterior part of a region where secretion had been active at the time that the specimen was fixed. A noticeable shreddy or feathery appearance is presented by the parts of the lamellae nearer to the outer muscular layer. The parts of the lamellae nearer to the inner epithelial layer have a quite different and more normal appearance.

The species thus far described, except *H. oculatus*, are often assigned to a subgenus Eisenia which is characterized by the position of the spermathecal pores near the median dorsal line. One other species of this subgenus which is common in North America and available for study is *H. roseus*. The calciferous gland of this species more closely resembles those of the group of species of which a description and discussion next follow.

ESOPHAGEAL POUCHES PRESENT; NO OTHER LATERAL ENLARGEMENTS

The other groups of species to be considered include those in which the anterior part of the calciferous gland has a pair of lateral evaginations of the esophageal wall, including cavities with which the wall chambers are in communication. This includes a majority of the species of Lumbricidae of which the glands have been studied. The species to first receive attention will be those in which there are no conspicuous definite lateral expansions of the gland wall posterior to the esophageal pouches, which is the term by which the evaginations will be designated.

Helodrilus palustris is an interesting North American species described quite fully by Moore (1895). He gave a detailed account of the calciferous gland and several excellent figures illustrating its anatomy. Dr. Moore kindly presented the writer with a few specimens several years ago, and these have furnished an opportunity to supply certain facts in addition to those found in his description. Moore noted the uniformity in diameter of this part of the esophagus, and the lack of pouches which are so conspicuous in Lumbricus, and which account for the custom of using the plural term "glands" in referring to it. His diagrammatic figure of a median sagittal section represents the anterior end of the gland as near the middle of somite 10, but his description implies that it is in the 11th somite. Figures 30 and 31 are from series of longitudinal sections of the writer's specimens and show clearly that the gland begins in 10. The epithelial layer is described as ciliated, but the figures of Moore and the writer's sections indicate that it is an example of "rodlet epithelium." At the anterior end of the gland there is an abrupt, definite, lateral expansion of the esophageal wall on each side of the esophagus and accompanying lateral extensions of the esophageal lumen forming cavities which are narrow antero-posteriorly, but more extensive laterally. These cavities communicate directly with the esophageal lumen and into them open the channels through which direct communication of these cavities with those of the wall chambers is established. They correspond in essentials with the cavities of the esophageal pouches or "first pair of glands" of such species as *Lumbricus terrestris* and will be considered as their equivalents, though relatively small in extent. The lamellae of the lateral walls are much wider than those of the dorsal and ventral walls and the esophageal lumen is reduced to a cleft posterior to the pouches, although the outer wall, as seen in transverse sections, is approximately circular in outline. Moore stated that he had not "found the rifts, or communications, between the follicles and the esophageal lumen, which Claparède observed in Lumbricus." Moore makes no reference to communications at the anterior end of the gland between the follicles (wall chambers) and the lumen and presumably thought that there were none. The writer's sections show narrow tubular channels lined with cilia and connecting the anterior ends of several of the chambers with the cavities at the anterior end of the gland, which open into the esophageal lumen. One such connecting tube is represented in figures 31 and 32. No communications have been found at the posterior end. There is a definite increase in the diameter of the gland over that of the esophagus, as shown in figure 30, and the chambers are much more narrowly wedge-shaped than are those of *H. oculatus*, but nevertheless the gland in *H. palustris* is an interesting intermediate type between that of the former species and certain more highly differentiated ones to be described later.

Helodrilus gieseleri (Ude) was originally described from specimens collected in Florida and the variety *H. g. hempeli* Smith from specimens collected in Illinois. The anatomy of the calciferous glands in these forms is very similar to that which is found in several closely related species belonging to the North American group of earthworms for which Michaelsen (1900a) established the subgenus Bimastus.

The gland has paired esophageal pouches in 10 but no special paired enlargements in 11 and 12 (Fig. 33). The chambers and separating lamellae, characteristic of the gland wall in somites posterior to 10, extend forward into the posterior part of the pouches in 10, and the lamellae are continued still farther forward as folds which are united at their base with the outer walls of the pouches, but with their inner margins disconnected and projecting freely into the pouch cavities (Fig. 34). Figure 35 is from a section slightly posterior and 36 from a section still farther posterior to the one figured in 34. They show that communication of the cavities of the wall chambers with the pouch cavities is afforded through openings arranged in a series along the outer wall of the pouches and ranging from the dorsal to the ventral parts of the same. The differences in relations shown in the two pouches is due in part to the sections being somewhat oblique, and perhaps in part to differences in the state of contraction, or of pressure of the surrounding organs. Figure 37 is from a sagittal section through the anterior end of the gland and near the lateral wall. It shows clearly the communications between the pouch and chamber cavities. Figure 38 is from a similar section of a specimen of the variety *hempeli*; the other figures being from a specimen of the typical form of the species. The chambers are approximately 40 in number and extend into somite 14 where they are much narrower than in the more anterior somites.

Helodrilus beddardi (Michaelsen) is another species originally described from North American specimens and found to range over a considerable part of the continent. It has also been reported from Asia by Michaelsen (1910) who has expressed doubt concerning its being specifically distinct from *H. parvus*. The general characters of the calciferous gland conform with those already described in *H. gieseleri*; definite esophageal pouches in 10; the lamellae and wall chambers wider in the anterior somites of the gland, but without definite conspicuous expansions like those of *Lumbricus terrestris* (Fig. 39). The number of wall chambers is approximately 40 throughout the major part of the gland. Figure 40 is from a transverse section through the pouches at the anterior end of the gland in a specimen collected in an acid bog. The lamellae folds are relatively wider than in ordinary specimens.

Helodrilus parvus (Eisen). This species was described from specimens collected in North America over which continent it is widely distributed. It has also been recorded in Africa and in different parts of Asia. The calciferous gland of this species was described in detail by Stephenson and Prashad (1919) with a figure from a transverse section through the esophageal pouches in 10.

Several series of sections from specimens collected in different parts of the United States have been available for study and an examination shows general conformity of the gland characters with those described in this species by Stephenson and Prashad and with those described above in the species *H. gieseleri*. The chambers and separating lamellae, characteristic of the wall of the gland in somites posterior to 10, are continued forward into the posterior part of that somite and the lamellae are continued still farther forward as folds which are united at their base with the outer walls of the esophageal pouches, but with the other margins of the lamellae disconnected, and projecting freely into the pouch cavities. Figure 44 is from a frontal section of a specimen of this species and shows two features that deserve mention. The dilation of the lumen in somite 13 is due to the lodgment at that spot of a mass of food material at the time the specimen was killed. The esophageal pouches present an appearance which is duplicated in one other specimen of which sections were examined. The pouches are more commonly dilated and saclike, extending farther out into the coelomic space.

Helodrilus roseus (Savigny) is a species which is widely distributed and found in all the continents and in most regions where Europeans have settled. The calciferous gland of this species was figured and described by both Ribaucourt and Combault. The latter author erred in the position of the anterior end of the gland and in the number of somites involved. A pair of esophageal pouches in 10 very definitely determine the position of the anterior end of the gland. There are no definite lateral expansions of the gland wall in somites posterior to 10 comparable to those in *Lumbricus terrestris* (Fig. 54) or in *Helodrilus lönnbergi*. The wall chambers and separating lamellae are continued into the posterior half of the esophageal pouches and there the cavities of the pouches and of the chambers are in direct and obvious communication through a series of openings between the ends of the lamellae and situated in close relation to the peripheral wall of the pouches (Fig. 64). The width of the lamellae and chambers of the lateral walls is relatively considerably less in this species than in most of those described, and the width of the esophageal lumen in the frontal plane is correspondingly greater.

Helodrilus tetraedrus (Savigny) is widely distributed in various parts of the world and has several forms or varieties which have been recognized. An abundance of material has been available for study, both of the typical form which has spermiducal pores on somite 13, and of the form or variety *H. t. hercynius* which has the spermiducal pores on 15. Both forms are found in abundance in the banks of a small stream which flows through the campus of the University of Illinois. Other specimens of the typical form from the Puget Sound region, from Colorado, and from several of the North Central states have also been available for comparative study. In the preparation of a paper on the Lumbricidae of North America several years ago (Smith, 1917), the writer's attention was attracted by the very poorly developed condition of the gland in some specimens, while a much more nearly normal condition was present in others. The thought that perhaps a more primitive condition of the organ might be represented in this species led to the preparation of sections of young specimens from cocoons, in the hope that they might throw light on the mode of origin of the gland. Instead of finding a series of folds appearing first and later a series of chambers formed by the fusion of the inner margins of the folds as was anticipated, the chambers were formed as tunnellike tubes from the beginning. It now seems more probable that something akin to a retrogression or degeneration of the organ is taking place in some specimens, at least. Some idea of the differences in the appearance of the organ in different individuals can be gained by a comparison of figures (45 and 46) from one specimen and figures (47 and 48) from another individual from the same locality.

The esophageal pouches in 10 are much larger in specimens which have the more extensive development of the longitudinal chambers (Fig. 47) and are smallest in specimens having the smallest chambers (Fig. 45). Something which may perhaps be reasonably construed as evidence of a sort of degeneration of structure, and which is found in each of the various specimens examined by the writer, is the presence of certain irregular cavities in close relation to the posterior part of the paired pouches in somite 10, and to the anterior ends of the longitudinal chambers in the esophageal wall near 10/11 (Fig. 55-57). Instead of there being a continuation of each of these chambers into the esophageal pouches in 10 and a direct communication there with the lumen of the esophagus, we find that most of the chambers and their separating lamellae terminate in these irregular cavities which in turn communicate with the pouch cavities through channels few in number when compared with the number of longitudinal chambers.

Peculiarities of the gland found in specimens showing different types of abnormalities will receive attention in another part of this paper (page 37).

Ribaucourt (1901) and Combault (1909) both gave attention to this species and referred to the lack of lateral enlargements of the gland posterior to the evaginated pouches in 10 ("diverticules de Perrier"). Combault's figure 22 which purports to be from a transverse section of the gland of this species represents the chambers as relatively much larger and fewer in number than they have been found in any of the writer's specimens. Ribaucourt's figure represents the chambers in a part of a section and indicates a number and shape of chambers much more nearly like those found in the American material.

Helodrilus chloroticus (Savigny) is a widely distributed species which has been found in large numbers in the banks of a stream in Urbana during the past few years, although it has not previously been reported in Illinois.

The pouches in somite 10 are more elongate, and their long axes more nearly approximate parallelism with the long axis of the esophagus than in most species (Fig. 49). Figure 50 is from a sagittal section and shows unusually well the continuity of each of the chambers through several somites, and of their cavities with that of the pouch to which they are related. Ribaucourt gives a brief description of the gland of this species and has a figure (1901, Fig. 60) which indicates rather elongate pouches, but is lacking in details.

Helodrilus caliginosus trapezoides (Dugès) is the most abundant and widely distributed of the earthworms found in the United States. The type form of the species together with the variety are collectively more abundantly represented in Europe than is any other species. They are found in most parts of the world where Europeans have settled.

The calciferous gland of the type form of the species has been described by Ribaucourt, Combault, and Stephenson and Prashad; and that of the form *trapezoides* by the first-named author. Combault gave a brief account and a figure of the gland of a form which he designated as *H. trapezoides*, but it is very different from any seen by the writer in specimens of that group.

A pair of large esophageal pouches are formed in 10, and on their posterior walls are found the anterior ends of the lamellae and chambers which are contained in the wall of the somites 11-14. A series of openings between the anterior ends of the lamellae in close relation to the peripheral walls of the pouches afford communication between the chambers and the pouch cavities as in several species described above. The number of wall chambers is usually 60 or more, and the lamellae separating the lateral chambers are relatively wide, with the result that the esophageal lumen is reduced to a rather narrow vertical cleft (Fig. 51). The vertical height of the lateral wall chambers is but a small fraction of the width in the plane of the lamellae and the cavities are narrow elongate clefts, widest towards the outer wall. In the outer and wider part of some of the chamber cavities, are longitudinal folds comparable to the outer parts of the lamellae in structure and in relations to the esophageal wall, but having their inner margins free. The width of these folds is highly variable but some of them increase in width posteriad and become lamellae with a resulting increase in the number of wall chambers. From about 60 or a few less near the anterior part of the gland, the number of chambers may increase posteriad to 65-70, there being individual variation in the numerical relations. The width of the lamellae separating the lateral chambers is somewhat reduced at the levels at which the septa join the gland wall, with an accompanying diminution in the diameter of the gland, but the variations in the dimensions of the different parts of the gland are not nearly as extensive as those in *Lumbricus terrestris* or in such forms as *Helodrilus lönnbergi*.

Octolasium lacteum (Örley) is a species which is widely distributed in both Europe and the United States and is reported from other parts of the world where Europeans have settled. The calciferous gland was studied by Ribaucourt (1901) who interpreted it as equivalent to "la glande antero-posterieure IV" of the components of the gland as understood by him. He found nothing which he interpreted as equivalent to "les diverticules de Perrier." His figures indicate an organ very different from that found by the writer. A description of the latter follows. Combault has a figure of the gland which was probably based on one of the figures of Ribaucourt. It is on a larger scale and more diagrammatic.

The writer's first study of the gland of this species was based on Illinois specimens and the results were so different from those of the two writers above named that a study of European material seemed highly desirable. Fortunately specimens from Hamburg which had been identified and presented to the writer by Dr. Michaelsen were available and sections of two of them were made and studied with the result that marked similarity was found in the Illinois and European material. Perfectly definite esophageal pouches are formed in 10 in both the Illinois (Fig. 52) and European (Fig. 53) specimens. The wall chambers and separating lamellae are continued on the posterior and lateral walls of the pouches in a manner closely similar to that described in *Helodrilus gieseleri*, and the relations shown in certain sagittal sections from the part of the gland near its outer wall resemble closely corresponding sections of *H. chloroticus* (Fig. 50). Posterior to the esophageal pouches the gland extends through somites 11-14. The diameter is somewhat greater in 11 than in following somites, but there are no conspicuous enlargements like those of Lumbricus. The chambers of the lateral wall are wide enough to reduce the esophageal lumen to a narrow vertical cleft in the anterior somites. Of two specimens from which transverse sections were prepared, one had 46 chambers in 11 and the other one had 54. Serial sections from seven specimens of which two were from Europe were studied and very close agreement was found among them all.

It is somewhat difficult to account for the discrepancy between the above account and that of Ribaucourt who considered the gland in this species to be comparable to that of *H. oculatus* in the absence of esophageal pouches; and to be of a primitive type, though having thicker walls than had the gland of the last named species. The much enlarged lumen in the anterior part of the gland which is represented in his figure 62 and in that of Combault (1909, Fig. 21) suggests the possibility that the specimen studied may have happened to have had that part of the esophagus distended by a food mass which altered the general appearance. Deformations from such a cause are often encountered.

Sections from a specimen of *Octolasium transpadanum* (Rosa) from Europe have been available for study and an examination of sections through the gland shows that in this species there are perfectly definite esophageal pouches similar to those described above in *O. lacteum*.

There are several other North American species which have glands which are similar to those of such species as *Helodrilus gieseleri* and *H. beddardi*, and hardly merit separate descriptions and illustrations. Among such species are *H. tenuis*, *H. subrubicundus*, *H. zeteki*, and *H. longicinctus*. The glands of the two species last named contain about 60 wall chambers and the others about 40.

ESOPHAGEAL POUCHES AND OTHER LATERAL ENLARGEMENTS PRESENT

The species of a third group remain to be considered and are those which like the widely known *Lumbricus terrestris* have a type of gland which includes well differentiated esophageal pouches and in addition has conspicuous paired enlargements of the lateral chambered wall in each of somites 11 and 12. Several species of Lumbricus and also *Helodrilus octaedrus* are included in this group. This is the type of gland which has led to the frequent statements in text-books and elsewhere concerning the presence of three pairs of calciferous glands in the common earthworms.

Lumbricus terrestris Linnaeus. In view of the repeated references to the gland of this species in the earlier parts of this paper, a brief memorandum of the most important points will suffice. The esophageal pouches in 10 are large and definite with obvious openings into the lumen of the esophagus which are but little anterior to the septum 10/11. On their posterior and lateral walls are found the anterior ends of lamellae and wall chambers and the cavities of the latter communicate here with the pouch cavities. In each of the somites 11 and 12 the lateral portions of the chambered wall are much enlarged. Transverse sections from the middle of either of these somites show that the lateral lamellae and included chambers are much wider than in the neighboring somites or at the parts of the gland where the septa are attached. The relations are quite similar to those in the corresponding somites of *Helodrilus lönnbergi* (Fig. 25). In 13 and 14 the normal dimensions are regained and the gland is of more nearly uniform diameter. The number of chambers is commonly 60 or more with additional folds or partial lamellae attached to the wall in the outer margins of some of the chambers. In no case has the writer been able to find anything corresponding to the openings at the posterior end of these chambers which Harrington and Combault believed to be present and his observations corroborate those of Stephenson and Prashad.

Specimens of *Lumbricus rubellus* Hoffmeister and *Helodrilus octaedrus* (Savigny) from the United States and of *Lumbricus castaneus* (Savigny) from Denmark have been available for study and are found to agree in important characters of the calciferous glands with the description given above for the gland in *L. terrestris*. Ribaucourt reached similar conclusions from an examination of European material of *L. castaneus* and *H. octaedrus*.

CALCIFEROUS GLANDS IN ABNORMAL EARTHWORMS

An examination of a considerable number of abnormal specimens belonging to several species of Lumbricidae has resulted in finding that with certain types of irregularities in the other organs there are correlated certain abnormalities in the calciferous gland. Bilateral asymmetry in the positions of the various organs on the right and left sides of a specimen is a type of abnormality very frequently encountered. Two recent papers by Green (1923) and Smith (1922) contain accounts of three such specimens belonging to as many different species. In normal specimens of Lumbricidae in which esophageal pouches are formed, they are in the same somite (10) with the anterior spermaries (testes) and spermiducal funnels, and also with the "hearts" of the penultimate pair. Irregularities of metamerism that lead to inequalities in the numbers of somites on the two sides of a specimen, such for example as somite 10 on one side being opposite 9 or 11 of the other side, will usually be accompanied by asymmetry; the spermary, esophageal pouch, etc., belonging to 10 of one side will be opposite organs belonging to 9 or 11 of the opposite side. The appearance of such irregularities is perhaps due in part to the fact that each of the two lateral halves of the earthworm has its own developmental center. Although the two sides more commonly develop their quotas of any particular somite at the same time and of the same size, they do not necessarily or always so do.

Another type of irregularity is due to the tendency in occasional specimens to form supernumerary organs, as illustrated by the specimens described by Green (1923). She suggests in partial explanation that some disturbance in developmental processes has led to the development of two somites with contained organs from each of some of the units of developing tissue which would normally give rise to but a single somite. In specimens of this kind, more than one esophageal pouch may be formed on the same side of the specimen.

Figures 58 and 63 are from sections of two specimens with asymmetrical internal organs, but with normal metamerism, in so far as the septa and segmentation of the body wall are concerned. *Helodrilus tetraedrus* is frequently represented by specimens, some symmetrical and others asymmetrical, in which the contained organs are in somites other than the normal ones. Some of the varieties of this species which have been described without a study of the internal organization have presumably been based on such specimens.

An abnormal calciferous gland of an unusual type is found in an adult twinned specimen of *Helodrilus tetraedrus* which was described in a recent paper (Smith, 1924). The specimen is an example of twinning with a ventral type of union, and its general characters were summarized as follows: "The general organization is similar to what might be expected if one should succeed in splitting the anterior 45 somites of a normal individual in a sagittal plane from the mid-ventral surface as far as the middle of the lumen of the alimentary tract; then in spreading the cut surfaces apart and inserting a specimen similarly treated which had a corresponding length and number of somites (placing the cut surfaces in contact), thus producing a specimen with a double number of setae, reproductive organs, nerve and vascular trunks and nephridia, but with a single alimentary tract and mouth.

"The double or twinned condition prevails in the anterior 45 somites and is followed by about 70 somites of the type of organization found in the posterior part of a normal individual."

Paired evaginated pouches (Fig. 59 and 61) and related longitudinal chambers were present in the part of the esophagus derived from the larger individual, while the part which was derived from the smaller individual had paired invaginations in somite 10 (Fig. 60). One of the pouches of the larger individual was filled with calcareous concretions (Fig. 59) while the other one had none. The specimen was somewhat asymmetrical so that the two organs of a pair do not show in the same section. Figure 62 is from a section through the gland region in 11 and shows chambers of a type in which there is less reduction than in some normal specimens. It also shows sections of the two dorsal vessels, the two ventral vessels, and the two pairs of latero-longitudinal vessels which the twinned individual possessed.

MODE OF ORIGIN OF CALCIFEROUS GLANDS

Reference has already been made on page 16 to the peculiar views expressed by Harrington, and later advocated by Combault, in which the part of the gland posterior to 10 was assumed to have its origin in the greatly modified vascular layers of the esophageal wall; and that only the pouches in 10 are "lined with true epithelium and are developed as diverticula of the esophagus." Also on page 20 reference is made to the arguments of Stephenson and Prashad in support of the older view that the gland had its origin in a series of longitudinal folds involving the epithelium, and that these folds became higher and their inner edges fused, resulting in the formation of longitudinal tunnellike chambers. No such series of developmental stages is known among Lumbricidae of the present day. Such a series does exist however within a single genus of another family, the genus Diplocardia, and it was the discovery of species exhibiting the culmination of such a series that has led to the preparation of this paper.

The genus Diplocardia is peculiar to North America and has abundant representatives from the latitude of Northern Illinois to the Gulf Coast, and from the southeastern states westward nearly to the mountain states. At least one species is found in Mexico. About a dozen species are known and there are probably others.

Worms of the genus Diplocardia differ greatly from Lumbricidae, and among the differences the positions of certain organs may be mentioned. In Lumbricidae there is one gizzard in somites 17 and 18, in Diplocardia there are two gizzards, commonly in 5 and 6. In the former the clitellum is posterior to the spermiducal pores which are on 15, except in one form in which they are on 13; in Diplocardia the clitellum is anterior to the spermiducal pores which are commonly on 19 and rarely on 18 or posterior to 19. In Diplocardia the calciferous gland, when definitely formed, involves 14 and 15. In *D. communis*, the type species of the genus, and in other known species from the north central and western states, a few low longitudinal folds in 14 and 15 (Fig. 65 and 66) are all that represent a calciferous gland. In *D. michaelseni* from North Carolina (Smith, 1924a), definite folds in 14 and 15 are considerably higher (Fig. 67 and 68) than in the species from states farther north and west. In *D. eiseni* from Florida the folds in 14 and 15 are still higher (Fig. 69 and 70), some of them extending well towards the middle of the esophageal lumen, but the inner margins of the folds still remain unconnected with each other. In *D. floridana*, a new species from Florida described in another part of this paper, there are numerous folds in 14 and 15 which have the inner margins of the highest folds connected by an epithelial layer (Fig. 78 and 79) in the anterior part of the organ. The folds have become separating lamellae forming the walls of short chambers which communicate freely with the lumen posteriorly, but not anteriorly.

In *D. mississippiensis*, a new species from Mississippi, also described in this paper, we find a still more highly developed series of folds which by fusion of inner margins become converted into separating lamellae and wall chambers (Fig. 71-73) which extend throughout the greater part of the organ. In the great number of the chambers and in their great width in the radial direction, we have an organ closely resembling the part of the gland found in 11 and 12 of some of the Lumbricidae with highly developed glands.

In view of the fact that various stages in the development of such a complex structure are represented in different species of the same genus, living contemporaneously, it seems to the writer that strong support is furnished for the view that the complex glands in Lumbricidae have had a similar mode of development.

DESCRIPTIONS OF TWO NEW SPECIES OF DIPLOCARDIA

DIPLOCARDIA MISSISSIPPIENSIS N. SP.

Four specimens collected in the spring of 1921 by H. R. Reed at McNeill, Mississippi, in soil on which carpet grass was growing, were received from C. V. Piper of the Bureau of Plant Industry at Washington, D. C. The specimens had been placed directly into the preservative without the use of anesthetics or fixing agents, and consequently are more or less contorted and strongly contracted. Each of three specimens was split in the median sagittal plane through approximately 25 anterior somites, and one half removed for sectioning. Transverse sections were made of the part belonging to the specimen which is designated as the type. Sagittal sections were made of the part from another specimen which will be known as paratype No. 1. Both halves of the anterior 24 somites were removed from the third specimen, designated as paratype No. 2, and transverse sections were made of one and sagittal sections of the other.

External Characters

These worms are relatively small in size. The type specimen has a length of 90 mm. and a maximum diameter of 2.5 mm. in the anterior part and is somewhat smaller posteriorly. The two paratypes are 81x2.5 mm. and 65x2 mm.; the diameter in each case being the maximum, and in the anterior part. The number of somites in the three specimens, given in the same sequence, is 148, 152, and 130. Nothing definite can be stated concerning the color, because of the method of preservation, but there is nothing that indicates much pigmentation or conspicuous coloration.

The setae are rather closely paired, but there is some variability in the spacing found in different somites of the same specimen and a little difference between the averages of different individuals. Using a, b, c, and d to represent setae on one side of a somite, beginning with a for the ventralmost one, we can represent the relative distances between the setae of the type specimen by the formula: $aa:ab:bc:cd=10:3:12:5$. These figures are based on the averages of measurements of several somites. The two dorsalmost setae are nearly at opposite ends of a transverse diameter. In paratype No. 2, the setal distances are more nearly represented by the formula: $aa:ab:bc:cd=12:5:16:9$. The setae a and b of somites 8, 9, and 10 are much modified in character in the three specimens studied. They are much longer and more slender than the ordinary setae, and the distal part is nearly straight and has the surface elaborately sculptured (Fig. 76).

In paratype No. 2, the setae of the ventral pairs on 11 are also modified in the same manner. The ventral setae of 18 and 20 are highly modified (penial setae) and closely associated with the distal part of the ducts of the prostate glands. They are extremely slender, somewhat curved and near the tip a little more strongly curved, while the tip itself seems slightly swollen. There is no evidence of surface sculpture. None of the specimens were at the height of sexual activity when preserved and the type specimen is the only one which has a well developed clitellum. It includes somites 13-18 and is nearly as thick on the ventral side as on the dorsal. Genital papillae are evident in the four specimens in the following situations: Surrounding each of the pairs of modified ventral setae in the spermathecal region is an area of thickened glandular epidermis. In each of these areas there is a rather extensive glandular development between the circular muscle layer and epithelium, quite similar to that described and figured by Eisen (1900, Fig. 154) in *Diplocardia udei* Eisen and stated by him to also occur in *D. michaelseni* Eisen. In three specimens, including the type, there are three pairs of such papillae on 8, 9, and 10. In paratype No. 2, a fourth pair is present on 11 associated with the modified setae of that somite, previously mentioned. A short transverse papilla is associated with the oviducal pores on 14, and another transverse papilla includes the two ventral bundles on 21. Prominent paired papillae are associated with the penial setae on somites 18 and 20, and the two papillae lying on either side of the median line are connected by a nearly straight longitudinal groove.

The position of the most anterior dorsal pore could not be determined with certainty since they are not always recognizable in the uncut specimens, and the part that was sectioned sometimes did not extend quite to the mid-dorsal line. Nothing was learned of the position in the type specimen. In paratype No. 2, a dorsal pore is recognizable at 11/12, and traces of what may be another one at 10/11 are present. None was found anterior to this position in any of the specimens. Nephridiopores are near the anterior border of the somites and dorsad of seta line d. The most anterior ones are in 2 and are dorsad of d by a distance greater than that between seta lines c and d, while the others are dorsad of d by a distance less than cd. The paired spermiducal pores are near the anterior border of 19, and in the longitudinal grooves which extend between the genital papillae on 18 and 20. The prostate gland pores are paired on 18 and 20 and are in close relation with the modified ventral setae of those somites. The oviducal pores are paired on 14. They are near the anterior border of the somite and mesad of seta line a. The spermathecal pores are paired on somites 8 and 9 in each of the four specimens examined, and paratype No. 2 has an additional one on one side of 7. Those of somite 8 are located $\frac{1}{4}$ to $\frac{1}{3}$ of the length of the somite from the anterior margin and between setal lines a and b, but usually nearer to the latter. The spermathecal pores of 9 in the type specimen are in about the same location with reference to the setae of that somite, as are those of 8 to the setae of 8, being at a considerable distance anterior to them. In each of the other three specimens examined, the pores on 9 are but slightly anterior to the ventral setae of that somite.

Internal Characters

Septa 8/9, 9/10, and 10/11 are the thickest and nearly equally developed; 7/8 and 11/12 are approximately two thirds as thick and the others much thinner. Two gizzards are well developed in somites 5 and 6, as in most known species of the genus. The walls of the esophagus in somites 9-13 have numerous short folds which fill up much of the lumen. They are richly supplied with branches from the blood plexus in the wall. The most interesting feature of the alimentary tract is the part in 14 and 15 which is more modified and complex in structure than in other known species of the genus. Sections show the structure of the walls to be comparable to that found in the part of the calciferous gland which is contained in somites 11 and 12 of most Lumbricidae. Figures 71 and 72 show some of the details of this structure. The widest of the numerous radiating longitudinal folds are united along their inner margins by an epithelial layer which includes a narrow secondary lumen. Posteriorly the numerous longitudinal chambers communicate with this lumen (Fig. 73). Cilia are especially numerous and well developed on the epithelial walls of the chambers near the outlets and presumably aid in propelling fluids from the chambers into the lumen. The gland is supplied with an extensively developed blood-vascular system which has several connections with the dorsal vessel. The alimentary tract in 16 is of relatively small diameter, and in 17 is abruptly widened and forms the anterior end of the intestine. The typhlosole begins in or near 18 and extends posteriad. Its general appearance in the first few somites is represented in figure 74 which is from a transverse section of somite 24. It includes a thin median layer continuous with the blood-vascular plexus in the body wall, and on each side of this a thickened epithelial layer continuous with that which bounds the intestinal lumen, but with the component cells fully twice as high as those of the intestinal wall. The vertical part which corresponds to the ordinary earthworm typhlosole extends at least to the middle of the lumen and is continued on each side of its distal margin into a fold lying approximately at right angles to the first, having a similar structure and appearance, and the two lateral folds having a combined width fully equal to that of the median part to which they are attached. This modified type of typhlosole is present in approximately fifteen somites of the anterior part of the intestine. Posterior to this part there is a reduction of the lateral wings and the appearance is more nearly like that of the ordinary earthworm typhlosole.

The circulatory system conforms closely to that already known in *Diplocardia eiseni* (Michaelsen). The dorsal vessel is single in the greater part of its course, except in a few somites in the clitellar region, where it is doubled between successive septa. A pair of large transverse vessels or "hearts" is present in each of somites 10 to 13. They are of the dorso-intestinal type, as are those of other Diplocardia species which the writer has had an opportunity to study. The chief connection of the dorsal ends of the hearts is with the richly developed vascular system of the esophageal wall and not with the dorsal vessel. There are frequent and extensive connections between the dorsal vessel and the vessels of the esophageal wall. In each of somites 7, 8, and 9, are much more slender "hearts" of ordinary type connecting the dorsal and ventral vessels. Such connecting vessels are often called dorsal hearts. In other Diplocardia species studied by the writer there is a definite continuous supra-intestinal blood vessel extending from somite 9 to 13 or 14, along the dorsal side of the esophagus, and between it and the dorsal vessel. With this vessel the dorso-intestinal hearts of such species have their chief dorsal connection. In the species here described such a continuous supra-intestinal vessel has not been identified and the chief dorsal connection of such hearts seems to be with the vascular plexus of the esophageal wall. It is possible that such a vessel may normally be present and the difficulty in finding it be due to the injuries occasioned in splitting the specimen preparatory to removing the sand from the alimentary tract in preparation for section cutting.

The nephridia are of the meganephric type and the first pair is in the second somite. The narrowed parts of the nephridial ducts which terminate in the nephridiopores exhibit in this species, with the exception of the first pair, a marked uniformity in their course; and in the level, with reference to the seta line d, at which they traverse the body wall. In tracing the duct from the nephridiopore, which is ordinarily slightly dorsad of seta line d, one finds that the course is directly through the outer epithelial layer; then slightly ventrad and posteriad in the circular muscle layer to the level of seta line d; and then through the longitudinal muscle layer to the coelomic cavity where it joins the main nephridial mass. The course through the longitudinal muscle layer is ordinarily between the two more or less definitely distinct bands of longitudinal muscle fibres which farther posteriad in the somite separate from each other, and extend one dorsad and the other ventrad of the corresponding d seta. These relations have been studied in only about 25 anterior somites.

The reproductive organs have characters commonly found in the genus. Spermaries and spermiducal funnels are paired in each of somites 10 and 11 and the sperm ducts of either side unite in 19 just before they open to the exterior. Paired sperm sacs in 9 and 12 open through septa 9/10 and 11/12 into somites 10 and 11 as in other species. The prostate glands of the anterior pair open to the exterior on 18, but occupy a great deal of space normally belonging to 18 and 19. Similarly those of the posterior pair open on 20, but extend into 21. In each of the three specimens sectioned, the duct of each prostate gland has a length nearly equal to half of the diameter of the worm and a circular muscle layer forms an important part of the wall. The duct extends dorsad and anteriad from its attachment to the body wall and enters the gland mass near the anterior septum. After its entrance into the gland, the lumen is traced with considerable difficulty, because of its very small diameter and the disappearance of the wall layers except a very inconspicuous epithelium. From its union with the duct, each gland extends dorsad and posteriad and then ventrad along the posterior septum, and then through the septum into the next following somite and again dorsad, filling a considerable part of the space between the intestine and body wall. The gland cells form many groups massed together and the secretion seemingly finds its way through very small inconspicuous passages to the main duct, itself very small, which extends through the middle of the gland from near its free end to the muscular walled duct which is its outlet. The structure of these glands seems to be very similar to that described by Eisen (1900) in *D. michaelseni* and *D. udei*. Ovaries and oviducal funnels are paired in 13 and the oviducts in 14. There are paired spermathecae in each of somites 8 and 9 in each of the specimens sectioned and in one of them, on one side of 7, is a spermathecal pore and a duct in the body wall which have a size and appearance similar to those of the corresponding parts of other spermathecae. A similar asymmetry in spermathecal development is noted by Michaelsen (1894, p. 189) in *D. eiseni.* Each spermatheca includes a rather long duct with a narrow lumen which opens into a large sac. Attached to the duct, somewhat nearer to the sac than to the spermathecal pore, is a cluster of a dozen or more small thin-walled chambers which communicate with each other and through several channels are also in communication with the lumen of the duct (Fig. 75). One such channel may afford communication for several chambers. In two specimens these chambers are filled with sperm cells.

DIPLOCARDIA FLORIDANA N. SP.

One specimen collected at Monticello, Florida, was received by the writer in January, 1914, while it was still living, and was preserved in good condition for study. It was not at a stage of sexual activity when collected and hence certain characters of the reproductive organs presumably differ somewhat from those of sexually active specimens. The anterior 22 somites were split in the median sagittal plane and longitudinal sections were made of the right half and transverse sections of the left half.

External Characters

The specimen is 98 mm. in length and varies in diameter from about 3 mm. in the anterior part to slightly more than 2 mm. posteriorly. The number of somites is 159, but the last four somites have an appearance that suggests the possibility that regeneration may have been taking place.

The setae are all included in the ventral half of the worm, the dorsalmost ones being but slightly ventrad of the ends of the transverse diameter. There is considerable variation in the spacing of the setae in different somites, but the relative distances are approximately indicated by the following formula which is based on averages of measurements made on several different somites. aa:ab:bc:cd = 8:3:8:5. The ventral setae of 8 and 9 are modified, but their characteristics have not been satisfactorily determined. Four are undeveloped and the other four were oblique to the plane of sectioning and only fragments are represented in any one section. Those examined are approximately 50% longer than setae of the ordinary type and scarcely more than two thirds as great in diameter. The distal end is nearly straight and is sculptured with extremely fine markings, very different in appearance from those of the corresponding setae of *D. mississippiensis* and *D. michaelseni.* There is of course no certainty that these setae are the same as those present at the time of sexual activity. The ventral setae of 18 and 20 are very small, slender, and much modified, but nothing can be determined concerning their sculpture.

There is almost no development of the clitellum nor of genital papillae. On the ventral side of 8 and 9, near the spermathecal pores, there are traces of gland development and a slightly thickened epidermal layer which indicate that paired genital papillae are present on those somites at the time of sexual activity. There are indications that they resemble the papillae similarly located in *D. mississippiensis.*

The first recognizable dorsal pore is at 10/11. The nephridiopores are at the anterior margins and the most anterior ones are in the second somite. 39 of the nephridiopores of the anterior 23 somites have been located and exhibit an unusual degree of variability in their position. Three are in seta line c; one between seta lines c and d; four in seta line d; the remaining 31 are dorsad of seta line d, but at varying distances. Using the symbol cd to indicate the average distance between the setae c and d, the locations of the nephridiopores dorsad of seta line d are about as follows: eleven of them are dorsad of line d by a distance less than cd, eleven of them at a distance about equal to cd, and nine vary from a distance about one and a half times cd to three times cd. The spermiducal pores are on 19, near the anterior margin, and nearly in seta line b. Each is borne on a very small papilla in a longitudinal groove extending between the prostate gland pores of the same side of the worm. The prostate gland pores are on 18 and 20 and open immediately posteriad of seta b of the same somites. The oviducal pores are paired on 14. They are anterior to the ventral seta bundles and mesad of seta line a. The spermathecal pores are paired on 8 and 9. Those of 8 are somewhat anterior to the ventral bundles and those of 9 are between the setae of the corresponding ventral bundles.

Internal Characters

Septa 8/9 and 9/10 are the thickest and about equally developed; septa 7/8, 10/11, and 11/12 are nearly as strongly thickened; 6/7 and 12/13 are much thinner, though slightly more developed than the remaining septa.

The alimentary tract is similar to that of other species of the genus in most respects. Two well developed gizzards are present in 5 and 6. In 9 to 13, inclusive, the inner layer of the esophagus has numerous short longitudinal folds which are richly supplied with blood. In 14 and 15 and chiefly in the latter somite, there is a more elaborate type of calciferous gland than any thus far described in the genus, except the one in *D. mississippiensis*, and it is scarcely less highly differentiated than the one in that species. Figures 78 and 80 will aid in the description of its anatomy. As in the last named species, there are numerous longitudinal folds of the inner layer of a much widened part of the esophagus, and the inner margins of the widest of these folds are connected by an epithelial layer that forms a secondary lumen (Fig. 78). This condition is found in the anterior part of the organ. As one follows the organ posteriad it will be noticed that the epithelial lining of the inner lumen becomes discontinuous on its lateral walls (Fig. 79), and that the spaces between the folds of these walls are continuous with that of the inner lumen. Still farther posteriad (Fig. 80) there is increasing discontinuity of the inner edges of the folds and a corresponding increase in the extent of the continuity of the spaces between the walls, and the disappearance of the definiteness of an inner lumen. No communications of the spaces between the folds with that of the secondary lumen are found in the part of the gland anterior to that represented by figure 78. The alimentary tract of 16 is relatively small in diameter with a few low folds. In 17 there is a marked increase in the diameter of the alimentary tract, forming what may be considered as the anterior end of the intestine. The wall of the anterior part of the intestine has an almost continuous layer of blood which has frequent connections with the dorsal vessel. The typhlosole (Fig. 81) is much like that of *D. mississippiensis*. In sections of somite 50 and of somite 100, the two lateral folds are still conspicuous, attached to the distal margin of the median fold which corresponds to the more common form of typhlosole found in ordinary earthworms. This triplicate type of typhlosole extends much farther posteriad than it does in *D. mississippiensis*.

The dorsal vessel is double between consecutive septa in somites 6–25 and single posterior to 25. A distinct supra-intestinal vessel is developed in 10–13. There are paired dorso-intestinal hearts in 10, 11, and 12 which unite the supra-intestinal and ventral vessels. Each of these hearts has a very small branch which joins the dorsal vessel. Dorsal hearts which have their principal dorsal connection with the dorsal vessel and are smaller than the dorso-intestinal hearts are paired in 7–9.

The nephridia are of the ordinary meganephric type and the first pair, which are very small, are in the second somite. The narrowed part of the nephridial duct which terminates in the nephridiopore follows a fairly direct course through the body wall to the coelomic cavity and, since there is much variability in the locations of the nephridiopores with reference to the seta line d, there is a corresponding variability with reference to the seta line d in the levels at which the nephridial ducts traverse the longitudinal muscle layer of the body wall. But a small percentage of the ducts have their course between the two bands of longitudinal muscle fibers which are in contact in seta line d and which, in the immediate vicinity of the dorsal setae bundles, separate with one extending dorsad and the other ventrad of d.

Spermaries and spermiducal funnels are paired in each of the somites 10 and 11. The sperm ducts of either side extend posteriad between the circular muscle layer and the coelomic epithelium, and unite in somite 18. Paired sperm sacs are present in 9 and 12 and not in intervening somites. The prostate glands are present in 18 and 20. Those of 18 are confined to that somite and those of the posterior pair opening on 20 have a part of the glandular portion extending into 21. The glands are of reduced size but apparently have the same general type of structure as that described in *D. mississippiensis*. Ovaries and oviducal funnels are paired in 13 and oviducts in 14. Spermathecae are paired in 8 and 9 and are presumably smaller than at the season of sexual activity, and with accessory structures less highly developed. Apparently certain rudimentary bodies attached to the spermathecal ducts are the representatives of the thin-walled storage chambers described in *D. mississippiensis.*

D. floridana and *D. mississippiensis* are closely related to two other species from the southeastern part of the United States and with them form a group in which are found interesting structural deviations from the characters found in known species of other parts of the United States. *D. michaelseni* Eisen from North Carolina and *D. eiseni* (Michaelsen) from Florida are the two other forms under consideration. The interesting series of stages of increasing complexity in the folds of the inner layer of the esophageal wall of somites 14 and 15, culminating in the highly complex gland described in *D. mississippiensis,* which so closely resembles the calciferous gland of Lumbricidae has already been discussed. Other peculiarities of the group of species mentioned are the presence of specially modified ventral setae in the spermathecal somites and of a peculiar type of glands related to the outer ends of those setae. The location of the spermathecal pores in the somites bearing them tends to be farther posteriad in members of this group than in other species. The usual position of the pores in other species is near the anterior margins of the somites, while in this group some or all of the spermathecal pores are near the ventral setal bundles, or still farther posteriad. The structure of the prostate glands in these species differs somewhat from that found in most others. This has been mentioned in the discussion of these organs in the preceding descriptions, and has been discussed at length by Eisen (1900, pp. 181 and 188) and also noticed by Michaelsen (1894, p. 188). In all four of the species the dorsal vessel is double between septa in most or all of somites 6–25 and single in the greater part of its course.

Eisen's description of *D. udei* indicates that that species is allied to the group in the possession of modified setae and associated glands in the spermathecal somites, and in the structure of the prostate glands, but not in the location of the spermathecal pores, in the character of the esophagus in 14 and 15, nor in having the dorsal vessel double in a part of its course.

The known distribution of the representatives of these closely related forms is limited to the relatively small territory which includes a few of the states in the southeastern part of the United States. This seems to give some support to the suggestion of Michaelsen (1894) that Diplocardia is a genus which phylogenetically is relatively young.

SUMMARY

The earliest important contribution to a knowledge of the anatomy of the calciferous glands in Lumbricidae was a paper by Leo in 1820. His ideas of the relations of the paired lateral enlargements of the esophageal wall to each other and to the lumen of the esophagus were more accurate than those of any subsequent writer prior to 1887, except those expressed by Henle, who in 1835 endorsed the views of Leo, but added nothing of significance. By the use of more refined methods in technique, Claparède in 1869 added much to the knowledge of the minute structure of the glands, but failed to gain as correct ideas of the general relations of the parts, as those of Leo. Most later writers, except Claparède, seem to have been unaware of the content of Leo's paper and have credited the later writers with the discoveries made by Leo.

Relatively few species of earthworms have glands of the type which is found in *Lumbricus terrestris* and which is the basis for the statements frequently made concerning the presence of "three pairs of glands." A greater number of species have a type of gland with but one pair of lateral enlargements, and these are in the tenth somite and correspond to the first "pair" in such species as *L. terrestris*. Several other species are found without any paired enlargements or pouches in the tenth somite, although a few of these may have one or two pairs in the next following somites.

Two new species of Diplocardia from the Gulf States are described, and in each of them, in somites 14 and 15, is found a type of gland which in structure and complexity strongly resembles the gland in Lumbricidae. A comparison of the gland structure in various species of Diplocardia discloses an interesting series of degrees in complexity beginning with the condition found in species from the latitude of Illinois, in which only a few low longitudinal folds are present in somites 14 and 15. More numerous and higher folds are found in a species from North Carolina, and a still further increase in number and height of folds is found in a Florida species. In the two new species the development of the gland has progressed still further, the inner edges of many of the folds becoming joined by a continuous epithelial layer with a resulting secondary lumen and a series of longitudinal tunnellike chambers, included between this lumen and the outer wall from which the folds developed. In view of the fact that various stages in the development of such a complex structure are represented in the different species of a single genus, living contemporaneously, there seems to be strong support for the contention that the complex glands in Lumbricidae have had a similar mode of development.

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PLATE I

EXPLANATION OF PLATE I

The figures on this plate are all from drawings made from figures in papers of some of the earlier writers on the calciferous glands of Lumbricidae. Only a part of the lettering has been reproduced.

Fig. 1 *Lumbricus terrestris.* Dissection showing sperm ducts and position of apertures. See footnote on page 12. From Leo (1820), Fig. VI.

Fig. 2 *Lumbricus terrestris.* Dissection of anterior part of alimentary tract showing openings into esophageal pouches. From Leo (1820), Fig. VIII.

Fig. 3 *Lumbricus terrestris.* Dissection showing calciferous gland and anterior part of alimentary tract. From Leo (1820), Fig. VII.

Fig. 4 *Lumbricus terrestris.* Part of esophagus with attached calciferous "glands." From part of figure by Morren (1829), Fig. 1, Plate XI.

Fig. 5 "Lumbrici minoris." As above, Morren (1829), Fig. 2, Plate XI.

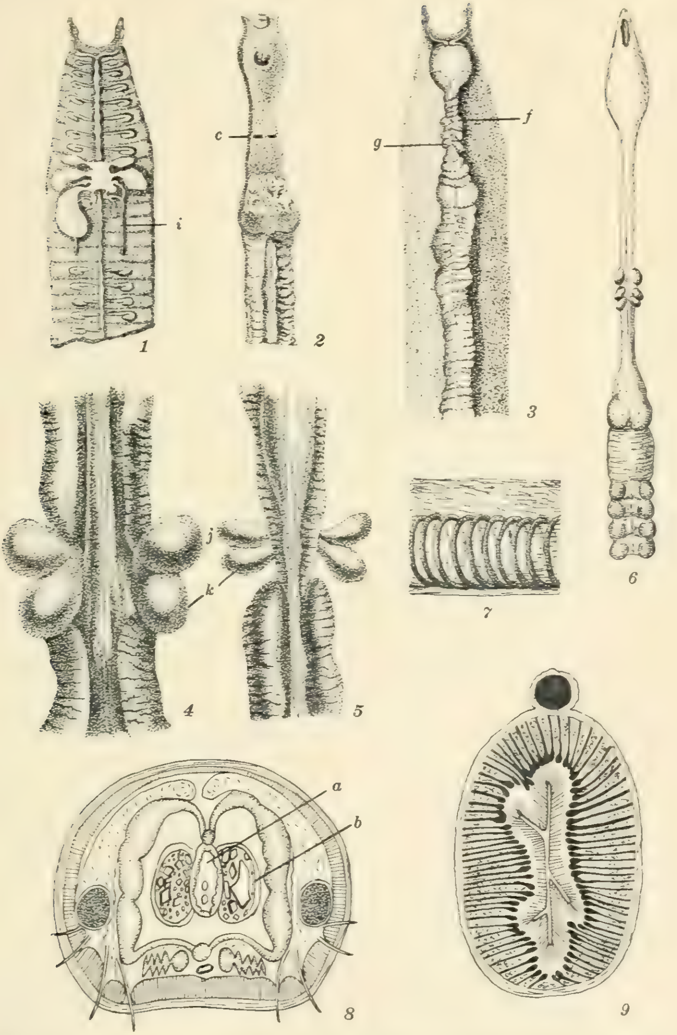
Fig. 6 Lumbricus. "Alimentary canal, removed from the other viscera." From Lankester (1864), Fig. 6, Plate VII. (*L. terrestris* and *L. agricola* were studied).

Fig. 7 Lumbricus. "Structure of oesophageal glands." From Lankester (1864), Fig. 2, Plate VII.

Fig. 8 *Lumbricus terrestris.* Transverse section passing through the esophageal pouches. From Claparède (1869), Fig. 1, Plate XLVIII.

Fig. 9 *Lumbricus terrestris.* Transverse section of gland, but not through enlargements in 11 and 12. From Claparède (1869), Fig. 1, Plate XLVI.

a	lumen of esophagus
b	esophageal pouch
c	openings of esophageal pouches
f	esophageal pouches
g	gland enlargements
i	sperm duct
j	anterior gland enlargements
k	posterior gland enlargements


i
1
c
2
f
g
3
6
j
k
4
5
7
a
b
8
9

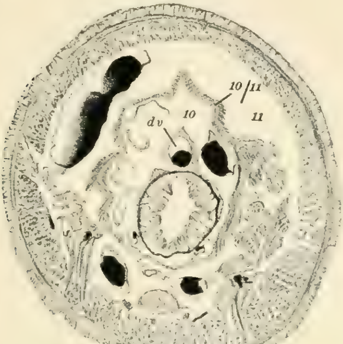
SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE I

PLATE II

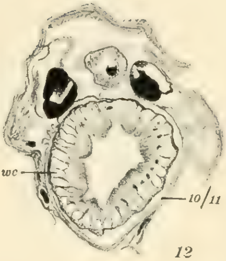
EXPLANATION OF PLATE II

Fig. 10 *Helodrilus oculatus.* Transverse section of worm, passing through the anterior part of the gland in 10, and showing a few lateral wall chambers. ×36.
Fig. 11 *Helodrilus oculatus.* Transverse section of esophagus, slightly anterior to gland in 10.
Fig. 12 *Helodrilus oculatus.* Section of gland at 10/11. ×57.
Fig 13 *Helodrilus oculatus.* Section of gland near middle of somite 11.
Fig. 14 *Helodrilus oculatus.* Section of gland at 13/14.
Fig. 15 *Helodrilus venetus hortensis.* Frontal section of gland in somites 11-13.
Fig. 16 *Helodrilus venetus hortensis.* Transverse section through the anterior part of the gland. ×112.
Fig. 17 *Helodrilus venetus hortensis.* Section through the gland at 11/12. ×112.

cc	connecting channel	*sec*	subepithelial cavity
dv	dorsal vessel	*wc*	wall chamber


10/11
10
11
dv

10



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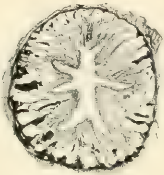

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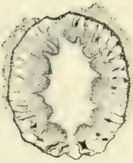

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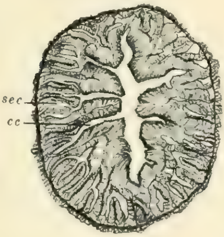

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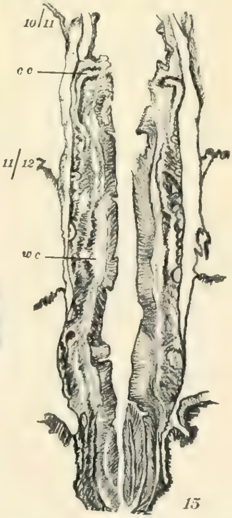
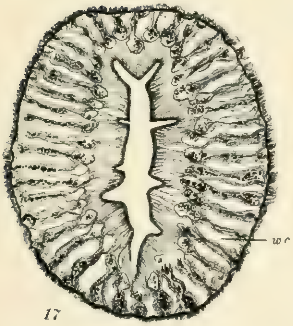

10/11
cc
11/12
wc

15



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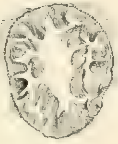


11

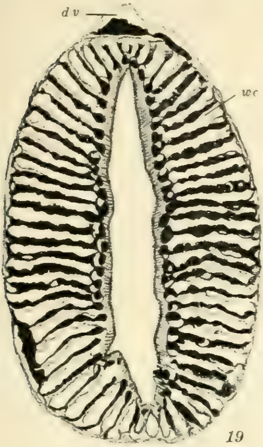
SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE II

PLATE III

EXPLANATION OF PLATE III

Fig. 18 *Helodrilus venetus.* Transverse section through the anterior end of the gland, and slightly oblique. ×36.
Fig. 19 *Helodrilus venetus.* Transverse section through the gland in somie 11. ×36.
Fig. 20 *Helodrilus foetidus.* Frontal section through the gland in somites 11-14. ×28.
Fig. 21 *Helodrilus foetidus.* Transverse section through the anterior end of the gland in somite 11.
Fig. 22 *Helodrilus foetidus.* Section slightly posterior to that of figure 21. ×56.
Fig. 23 *Helodrilus foetidus.* Section of gland in posterior half of 11. ×56.
Fig. 24 *Helodrilus foetidus.* Section of gland in somite 12. ×36.

cc connecting channel
dv dorsal vessel
lv latero-longitudinal vessel
sec subepithelial cavity
vv ventral vessel
wc wall chamber



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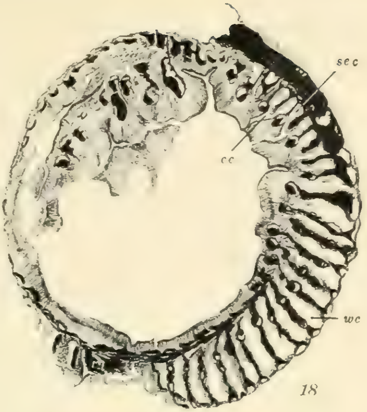

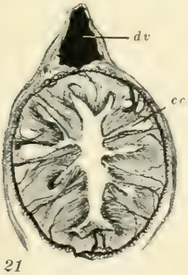

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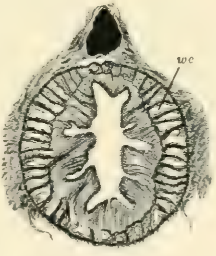

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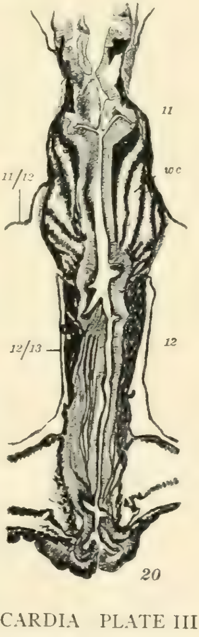
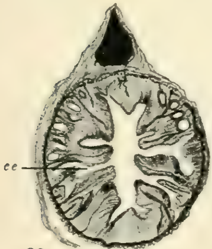

11
11/12
wc
12/13
12

20



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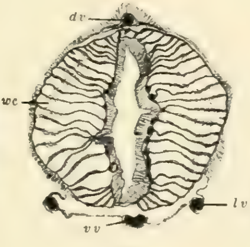


24

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE III

PLATE IV

EXPLANATION OF PLATE IV

Figures on this plate are all from sections of *Helodrilus lönnbergi.*

Fig. 25 Frontal section of anterior part of gland in somites 10-12. ×18.

Fig. 26 Frontal section of posterior part of gland in somites 13 and 14. ×18.

Fig. 27 Transverse section through anterior part of gland in somite 10. ×21

Fig. 28 Transverse section through the worm and passing through the gland in somite 11. ×11.

Fig. 29 Part of a section through the wall of the gland near the anterior part of somite 11 showing calcareous granules and gland structure. ×56

Figure 82 belongs with this series.

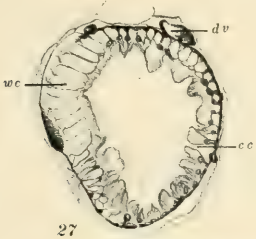
cc connecting channel

cg calcareous granules

dv dorsal vessel

l lamella

wc wall chamber



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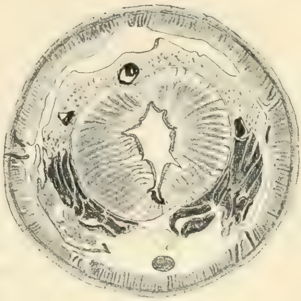

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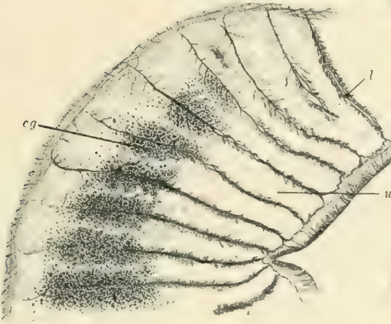

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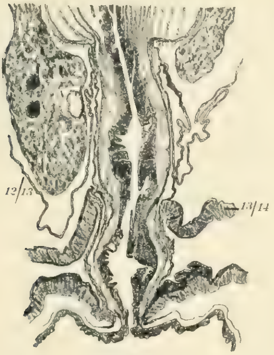

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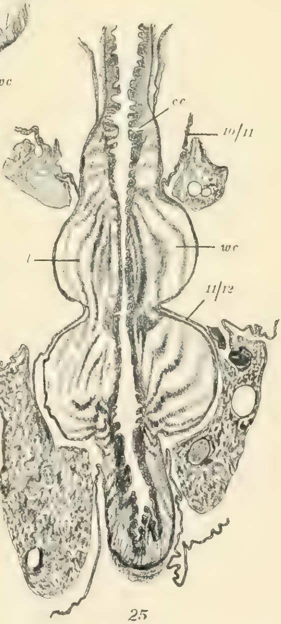

cc
10/11
wc
l
11/12

25

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE IV

PLATE V

EXPLANATION OF PLATE V

Fig. 30 *Helodrilus palustris.* Frontal section of anterior part of gland in somites 10-13. ×36.

Fig. 31 *Helodrilus palustris.* Sagittal section through chambers in gland wall in somites 10 and 11. ×56.

Fig. 32 *Helodrilus palustris.* Small part of section (Fig. 31) more highly magnified to show channel connecting the cavity of a wall chamber with that of the esophageal pouch. ×250.

Fig. 33 *Helodrilus gieseleri.* Frontal section of gland in somites 10-15.

Fig. 34 *Helodrilus gieseleri.* Transverse section through esophageal pouches near anterior ends of lamellae. ×36.

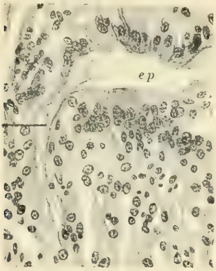
Fig. 35 *Helodrilus gieseleri.* Section slightly posterior to that of figure 34, showing a few chambers.

Fig. 36 *Helodrilus gieseleri.* Section still farther posterior, showing more chambers.

Fig. 37 *Helodrilus gieseleri.* Sagittal section through an esophageal pouch showing communication of pouch and chamber cavities. ×56.

Fig. 38 *Helodrilus gieseleri hempeli.* Section showing the same relations as in figure 37. ×90.

cc	connecting channel
ep	esophageal pouch
l	lamella
wc	wall chamber



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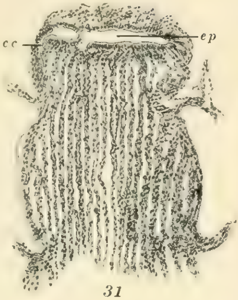

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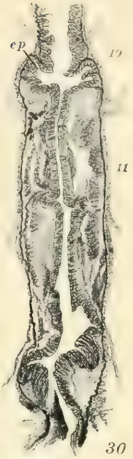

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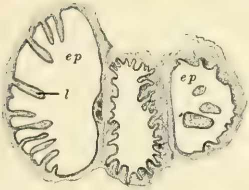

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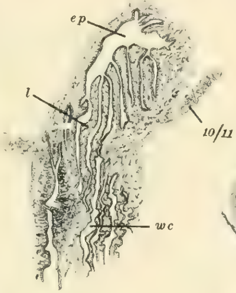

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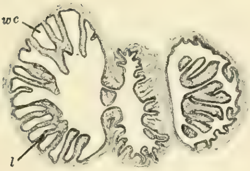

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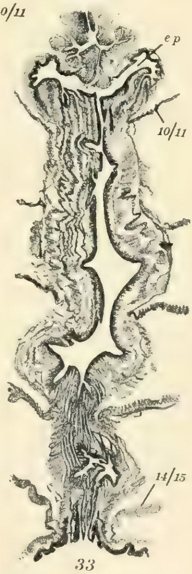
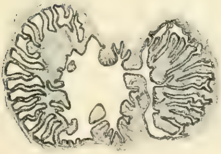

ep
10/11
14/15

33



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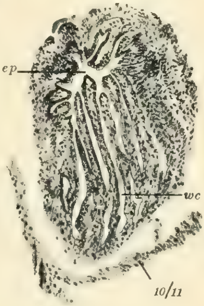


38

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE V

PLATE VI

EXPLANATION OF PLATE VI

Fig. 39 *Helodrilus beddardi.* Frontal section of gland in somites 10 to 14.

Fig. 40 *Helodrilus beddardi.* Transverse section through the esophageal pouches showing unusually wide lamellae and chambers. ×56.

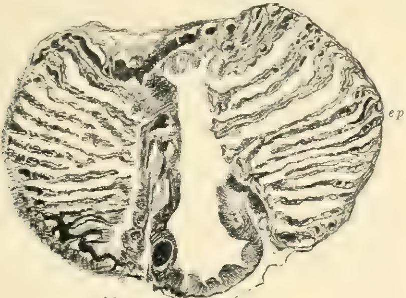
Fig. 41 *Helodrilus roseus.* Frontal section of gland in somites 10 to 14.

Fig. 42 *Helodrilus roseus.* Sagittal section through esophageal pouch near outer wall. ×48.

Fig. 43 *Helodrilus roseus.* Sagittal section from same specimen as above, but farther mesad. ×48.

Fig. 44 *Helodrilus parvus.* Frontal section of gland in somites 10 to 14.

ep esophageal pouch	*wc* wall chamber
l lamellar fold	



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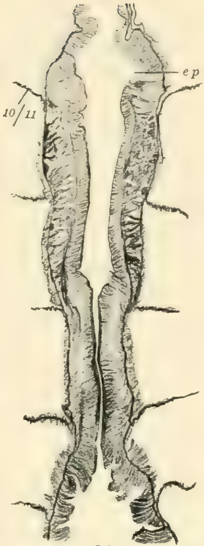

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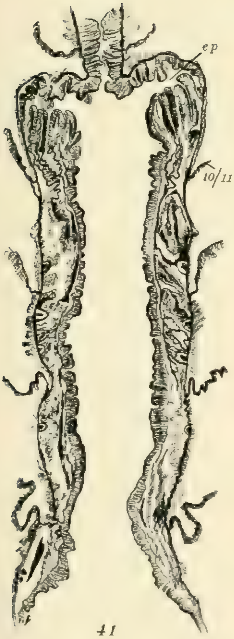

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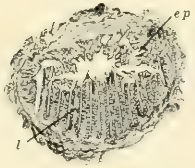

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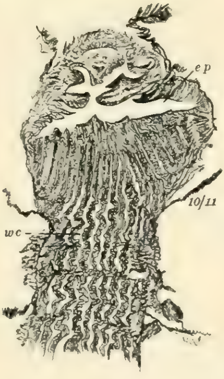

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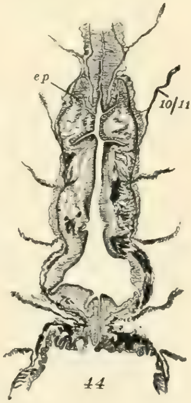


44

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE VI

PLATE VII

EXPLANATION OF PLATE VII

Fig. 45 *Helodrilus tetraedrus*. Frontal section of gland in a specimen in which the chambers are much reduced in width. ×28.

Fig. 46 *Helodrilus tetraedrus*. Transverse section of gland in a specimen similar to one in figure 45. Epithelium poorly preserved. ×56.

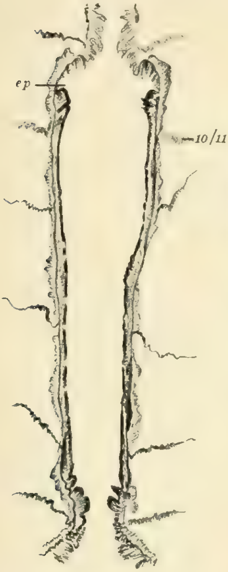
Fig. 47 *Helodrilus tetraedrus*. Frontal section of gland in a specimen in which the chambers are better developed than in the one in figure 45. ×28.

Fig. 48 *Helodrilus tetraedrus*. Transverse section of gland in a specimen similar to one in figure 47. ×56.

Fig. 49 *Helodrilus chloroticus*. Frontal section of gland in somites 10-14.

Fig. 50 *Helodrilus chloroticus*. Sagittal section of gland in 10-13, passing through chambers near outer wall and showing continuity of cavities of chambers and pouch. ×36.

ep esophageal pouch
l lamella
wc wall chamber



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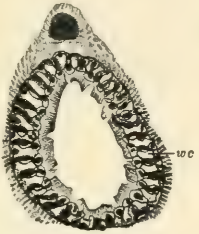

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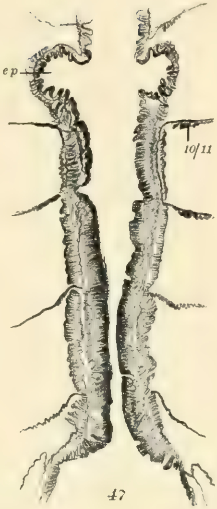

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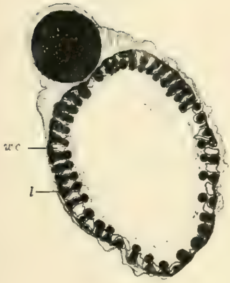

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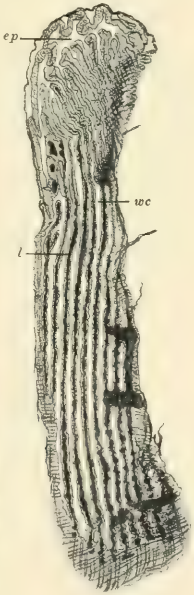

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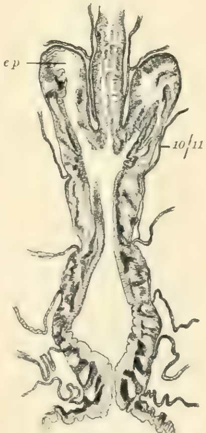

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SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE VII

PLATE VIII

EXPLANATION OF PLATE VIII

Fig. 51 *Helodrilus caliginosus trapezoides.* Transverse section of gland in somite 11. ×28.

Fig. 52 *Octolasium lacteum.* Frontal section of gland in somites 10-14. ×18.

Fig. 53 *Octolasium lacteum.* Frontal section of anterior part of gland in somites 10 and 11 in a European specimen which was strongly contracted. ×36.

Fig. 54 *Lumbricus terrestris.* Frontal section of gland in somites 10-15. ×11.

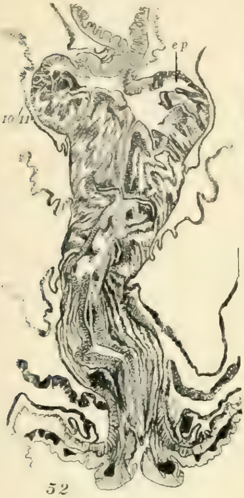
Fig. 55 *Helodrilus tetraedrus.* Transverse section of gland slightly posterior to esophageal pouch and through irregular cavity in wall. ×64.

Fig. 56 *Helodrilus tetraedrus.* Frontal section of gland, showing chambers entering irregular cavity in wall near the esophageal pouch. ×64.

Fig. 57 *Helodrilus tetraedrus.* Sagittal section of gland, showing similar structure to that mentioned in figure 56. ×64.

Fig. 58 *Helodrilus caliginosus trapezoides.* Frontal section of gland in abnormal specimen.

dv	dorsal vessel
ep	esophageal pouch
ic	irregular cavity
l	lamella
wc	wall chamber



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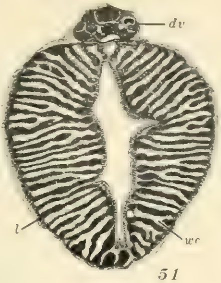

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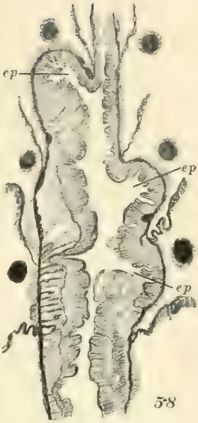

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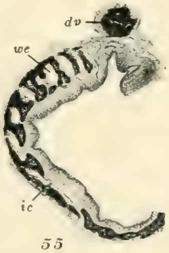

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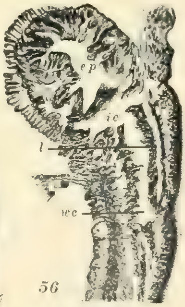

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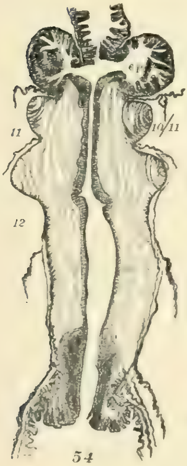
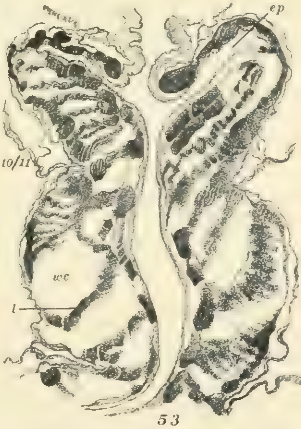

ep
11
10/11
12

54



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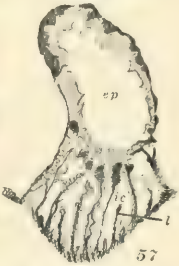


57

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE VIII

PLATE IX

EXPLANATION OF PLATE IX

Fig. 59 *Helodrilus tetraedrus.* Transverse section of twinned specimen, through esophageal pouch of one side of larger individual. ×28.

Fig. 60 *Helodrilus tetraedrus.* Section of gland of above specimen, through invaginated pouch of smaller individual and anterior part of chambered wall on one side of larger individual.

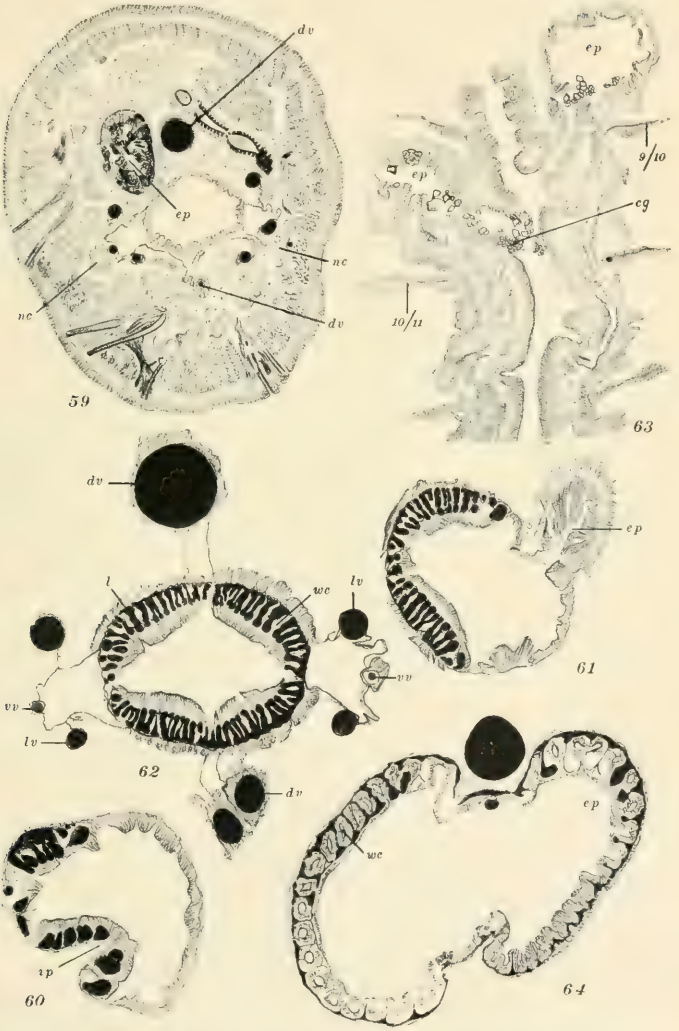
Fig. 61 *Helodrilus tetraedrus.* Section of gland of above specimen, through other esophageal pouch of larger individual and through wall chambers of opposite side in both individuals. ×56.

Fig. 62 *Helodrilus tetraedrus.* Section of gland of above specimen, showing wall chambers and lamellae of both individuals. ×56.

Fig. 63 *Helodrilus tetraedrus.* Frontal section through anterior part of gland in abnormal specimen with asymmetrical organs.

Fig. 64 *Helodrilus roseus.* Transverse section through the esophageal pouches near the anterior ends of the wall chambers. Transition from lamellae to folds and communication of chamber and pouch cavities are illustrated. ×68.

cg	calcareous granules
dv	dorsal vessel
ep	esophageal pouch
ip	invaginated pouch
l	lamella
lv	lateral vessel
nc	nerve cord
vv	ventral vessel
wc	wall chamber


dv
ep
nc
nc
dv
59
ep
9/10
ep
cg
10/11
63
dv
ep
l
wc
lv
vv
vv
lv
61
62
dv
ep
wc
ip
60
64

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE IX

PLATE X

EXPLANATION OF PLATE X

Fig. 65 *Diplocardia communis.* Transverse section through folds of esophagus in somite 14. ×36.

Fig. 66 *Diplocardia riparia.* Transverse section through folds of esophagus in somite 14. ×36.

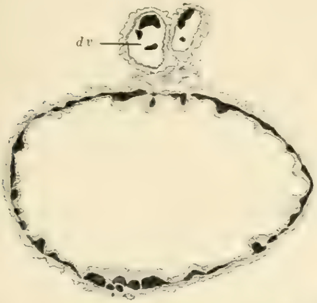
Fig. 67 *Diplocardia michaelseni.* Frontal section through gland in somites 14 and 15.

Fig. 68 *Diplocardia michaelseni.* Transverse section through gland in somite 14.

Fig. 69 *Diplocardia eiseni.* Frontal section through gland in somites 14 and 15.

Fig. 70 *Diplocardia eiseni.* Transverse section through gland in somite 14.

dv dorsal vessel



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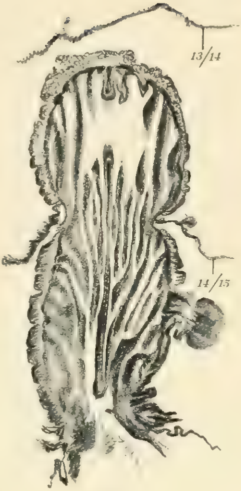

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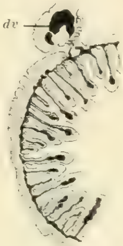

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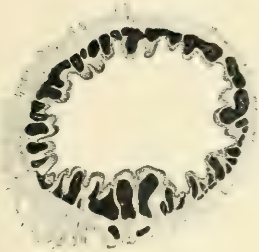

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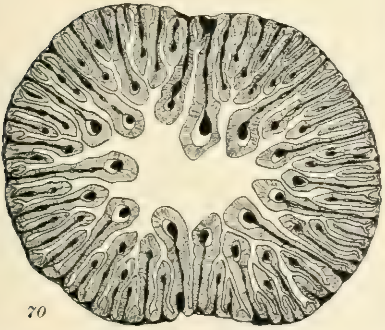

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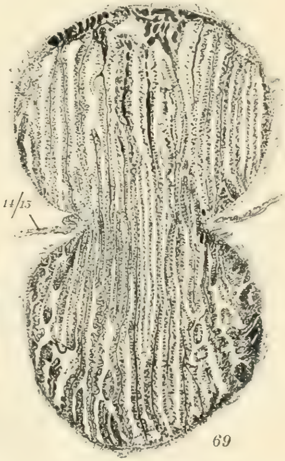

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SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE X

PLATE XI

EXPLANATION OF PLATE XI

Figures on this plate are all from *Diplocardia mississippiensis* n. sp.

Fig. 71 Frontal section through calciferous gland in somites 14 and 15.

Fig. 72 From a transverse section through median part of gland showing parts of gland and body wall and their relative size. ×35.

Fig. 73 From a transverse section through the gland near its posterior end. Only one side of gland is shown. ×48.

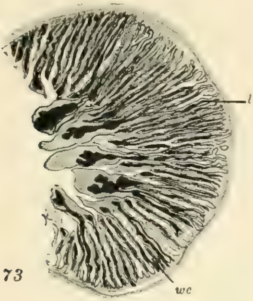
Fig. 74 Transverse section through the intestine in somite 24 showing the typhlosole. A part of the intestine had been removed. ×36.

Fig. 75 From a section through a spermatheca showing the accessory sacs attached to the duct. ×56.

Fig. 76 Transverse section, showing modified seta b of somite 8 and related structures. ×105.

Fig. 77 Distal end of modified seta b of somite 10.

bw	body wall	*sd*	spermathecal duct
ds	sacs of spermathecal duct	*sg*	setal gland
dv	dorsal vessel	*ss*	spermathecal sac
l	lamella	*t*	typhlosole
s	modified seta	*wc*	wall chamber



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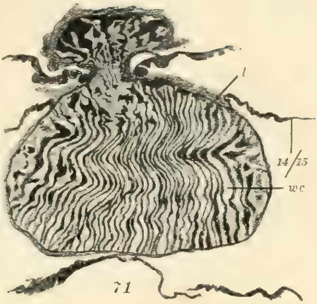

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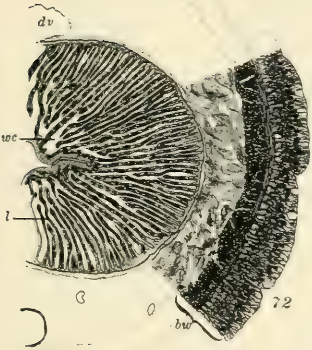

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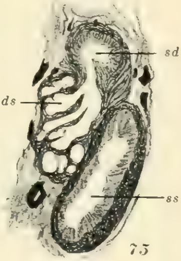

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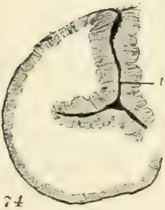

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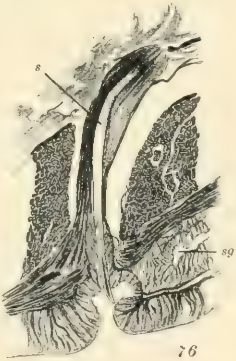

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SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE XI

PLATE XII

EXPLANATION OF PLATE XII

Fig. 78 *Diplocardia floridana* n. sp. Transverse section of part of wall of anterior part of calciferous gland in somite 14. ×56.

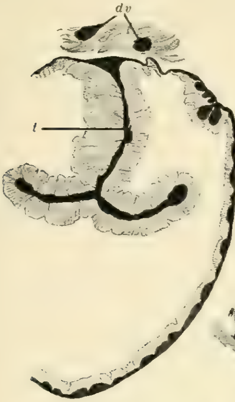
Fig. 79 *Diplocardia floridana.* Transverse section a little posteriad of the one in figure 78.

Fig. 80 *Diplocardia floridana.* Transverse section of gland near posterior end in somite 15.

Fig. 81 *Diplocardia floridana.* Transverse section of part of intestine in somite 22. ×36.

Fig. 82 *Helodrilus lönnbergi.* Frontal section through part of gland in an immature specimen, showing large size of gland in somites 11 and 12. This figure belongs with the series numbered 25-29. ×20.

dv	dorsal vessel	*t*	typhlosole
l	lamella	*wc*	wall chamber



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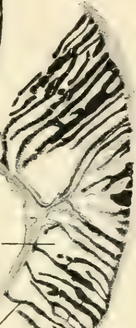

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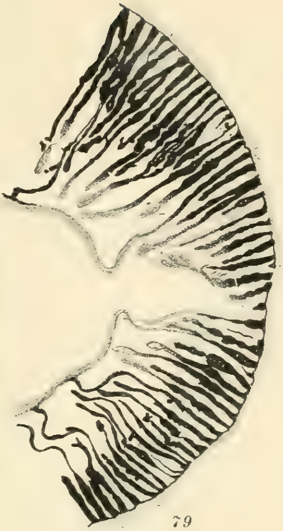

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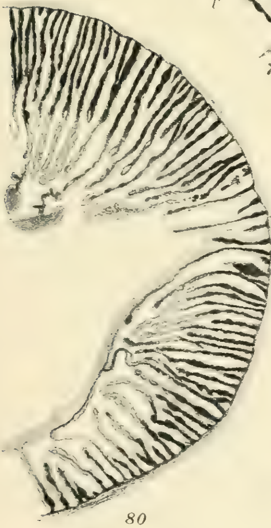

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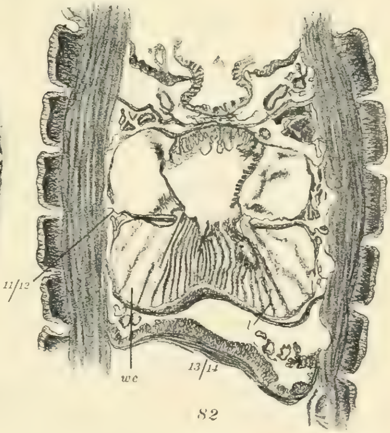
11/12
l
wc
13/14

82

SMITH GLANDS OF LUMBRICIDAE AND DIPLOCARDIA PLATE XII

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A BIOLOGIC AND TAXONOMIC STUDY
OF
THE MICROSPORIDIA

WITH 27 PLATES AND 9 TEXTFIGURES

BY
ROKSABRO KUDO

Contributions from the
Zoological Laboratory of the University of Illinois
No. 246

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PREFACE

For more than a century there has been known a microorganism which causes an infectious disease in the silkworm (*Bombyx mori*). As the attention of the biologists was directed toward it, it was revealed that similar organisms were widely distributed among various kinds of animals. The disease of the silkworm, known as pébrine and caused by an organism for which Nägeli proposed the name, *Nosema bombycis*, reached such an epidemic state in the latter half of the last century that the annual silk production of France and Italy was greatly reduced and that it threatened to wipe out completely the industry in those countries.

As a result several distinguished microbiologists among whom Pasteur and Balbiani stand out prominently, undertook investigations of the disease and its cause. The so-called pébrine organism thus became the center of attention for numerous biologists and physicians in European countries, and this resulted in finding numerous organisms of similar nature in a variety of host animals. In 1882 Balbiani proposed naming the pébrine organism and related forms, Microsporidia, and placing the group in the class Sporozoa.

Some years later Thélohan carried out very careful experiments and painstaking observations on the Microsporidia resulting in a splendid monograph (1895) on the group of the sporozoans here dealt with.

Labbé followed Thélohan in giving a synopsis of genera and species of Microsporidia in his Sporozoa (1899). In the last twenty years the efforts of various students of the Microsporidia have brought out numerous facts concerning the morphology and life history of these protozoans and have discovered many new and interesting forms. Part of these discoveries is included in Die Cnidosporidien, a valuable contribution by Auerbach (1911) who gave in it biological discussions and a brief taxonomic consideration of these organisms.

During the last decade, workers have brought to light facts on the organization of different phases in the development of Microsporidia and also have shown that these organisms are frequent parasites of some invertebrates which are closely associated with man.

In view of these circumstances it seems worth while to summarize the present state of knowledge on the Microsporidia. The objects of the present paper are in the main: 1) to collect in one paper for easy references all the published information on Microsporidia, now very widely scattered; 2) to review critically the facts observed or inferred by different investigators as to the morphology and development of the organisms; 3) to bring out the relationships between the Microsporidia and their hosts; 4) to review the zoological, geographical and seasonal distribution of the Microsporidia, and 5) to give a complete taxonomic survey of the species that are known at present. The text is divided into two portions. In the first half, the biology, I have attempted to deal with the first four objects, and in the second part, the taxonomy, each species is described as fully as possible with illustrations taken from the original papers.

INTRODUCTION

On the basis of cell-organs of locomotion and modes of living the Protozoa are divided into four groups which are usually called classes: Rhizopoda, Flagellata, Sporozoa and Ciliata. The class Sporozoa was named by Leuckart (1879). The sporozoans are characterized by their mode of existence and by possession of the so-called spores. Schaudinn (1900) subdivided the class into two subclasses: Neosporidia and Telosporidia, according to the time of sporulation.

The subclass Neosporidia will be divided here into the following superorders and orders:

Subclass Neosporidia Schaudinn 1900
  Superorder Cnidosporidia Doflein 1901 The spore possesses at least one polar filament and typically a polar capsule in which the polar filament is coiled.
    Order Myxosporidia Bütschli 1881 The spore contains one, two or four polar capsules, each enclosing a coiled filament, which are distinctly observable in the fresh state. The spore membrane is bivalve. The sporoplasm is single or rarely double. Parasites of lower vertebrates, particularly of fish (Kudo, 1920a).
    Order Microsporidia Balbiani 1882 The spore is minute and contains a relatively long polar filament that is typically coiled in a polar capsule which in many species cannot be seen in the fresh state. Each spore possesses a single sporoplasm. Cell-parasites of invertebrates, particularly of arthropods.
    Order Actinomyxidia Stolc 1911 The spore contains three polar capsules, each possessing a polar filament, with three shell-valves and numerous sporoplasms. Parasites of invertebrates.
  Superorder Acnidosporidia Cépède 1911 The spore does not possess either polar filament or polar capsule.
    Order Haplosporidia Lühe 1900
    Order Sarcosporidia Bütschli 1882
    Order Paramyxidia Chatton 1911

An interesting form was described by Keilin (1921) under the name of Helicosporidium. The spore which is peculiar in shape as well as in structure, contains three centrally located sporoplasms and a peripheral spiral filament and is surrounded by a thin spore membrane of a single piece. Helicosporidia thus would appear to be intermediate between Cnidosporidia and Acnidosporidia. The present paper deals exclusively with the order Microsporidia.

PART I BIOLOGY

THE SPORE

A microsporidian spore, although comparatively small, presents a typical appearance and possesses a characteristic structure. It is of interest from several different points of view. To biologists, it is of special interest on account of being one of the smallest animal cells. Yet it possesses a remarkable structure in its coiled polar filament. The filament is fine and is extremely long, in some cases reaching 50 times the length of the spore. Some authors suppose further that this filament is a hollow structure, the verification of which seems to be beyond the limit of the range of present optical apparatus.

Secondly, the microsporidian spores have as great power of withstanding unfavorable external conditions as bacterial spores and thus become the source of infection in new host individuals. Hence the spore occupies a premier place in practical studies of microsporidian infections. In the third place, it is this stage of a microsporidian which enables an observer to determine whether the microrganism he has in hand is a microsporidian or not and further to differentiate one microsporidian from the other. It is, of course, understood that the vegetative form has distinct characteristics and is important for a thorough comprehension of the organism; one, however, is lost if he does not see the spore stage.

The spore therefore will be considered somewhat in detail in the following pages. The study here is based upon the 139 so-called species of Microsporidia.

DIMENSIONS

Although microsporidian spores vary from 1.25μ long by 1μ broad (*Nosema pulvis*) to 17 to 23μ long by 3.5μ broad (*Mrazekia argoisi*), the majority are only 3 to 8μ long (Textfig. A). In almost all cases the spores of one and the same species occurring in a single host individual or cell vary in size to a greater or less extent, a fact which has been known since the days of early workers (Thélohan, 1895). As a rule, however, one finds intermediate forms between the two extremes. Most conspicuous polymorphism was noted in *Nosema marionis* (length 1.5 to 7μ), *N. pulicis* (2.5 to 5μ long), *Stempellia mutabilis* (length 2 to 6μ) and *S. magna* (12.5 to 16.5μ long). In the Microsporidia which I have studied, the difference in the dimensions of the spores seems to result from the difference in size of the schizonts and sporonts and further to differences in the process of sporogony, conditions which are quite probably due among many unknown circumstances to the environmental conditions in the host body—notably the size, nature and condition of the host cell in which the development of the microsporidian took place.

Several investigators have noticed dimorphism in the spores of many species. In an ambiguous form which he found in *Lyda nemoralis*, Kulagin (1898) noted bodies of two kinds. One was small spherical bodies about the size of the spores of *Nosema bombycis*, the other twice as big as the first form. Kulagin compared them with the microgametes and macrogametes of the Sporozoa, which is a mere supposition not supported by any evidence. In *Thelohania varians*, Léger (1897) saw macrospores measuring about 8μ long, in spherical masses in variable number, while the microspores, 4 to 5μ long, were grouped in eights and surrounded by a thin membrane. By finding dimorphic spores in *Plistophora mirandellae*, Vaney and Conte (1901) held that "les macrospores servent à la propagation de la maladie dans l'hôte et les microspores, par suite de la résistance de leurs kystes, probablement à la propagation hors de l'hôte."

Hesse (1903) notes in *Gurleya legeri* two kinds of pansporoblasts. The macrospore and microspore are essentially of a similar structure except for the polar filament: that is while the microspore possesses a polar filament, the macrospore does not have any. Mackinnon (1911) on the other hand states that "the large pansporoblasts occasionally hold only three spores, and these seem bigger than where there are the usual number. These spores would correspond to Hesse's macrospores, but I do not find the difference in size between the macrospores and microspores as great as he describes it in the parasite from Ephemerella." Hesse (1903a) noted both octosporous and tetrasporous pansporoblasts in *Thelohania janus*. These gave rise to microspores and macrospores respectively, the latter failing to extrude the polar filament under suitable treatment. Cépède (1911) noted a similar condition in the macrospore and microspore of *Gurleya richardi*. For *Thelohania fibrata*, Strickland (1913) reports similar conditions. I have noticed that in certain species of Thelohania, particularly *T. opacita* (Kudo, 1922, 1924c), tetrasporous and octosporous sporonts occurred frequently which resulted in the production of large and small spores. By pressure method, however, I always detected the presence of a filament in both spores. In *Stempellia magna* (Kudo, 1924b) which is mictosporous, I found four kinds of spores with reference to their dimensions. Schuberg (1910) recognized a distinct dimorphism in the spores of *Plistophora longifilis* with only difference in dimensions. Thus according to Hesse, and Cépède, the macrospores do not possess the polar filament, while Vaney and Conte, Schuberg and Kudo hold that the macrospore possesses the filament and the only difference between microspore and macrospore is their dimensions.

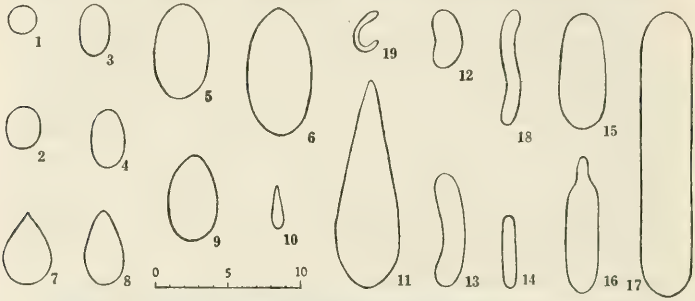
What are the underlying factors which cause dimorphism in microsporidian spores? In the polysporoblastic forms such as Thelohania, the large spores, macrospores, seem to be formed in the sporont whose nucleus divides only twice instead of three times as in the octosporous sporont and the latter is more abundant than the tetrasporous. Possibly the two species of Plistophora show dimorphic spores formed in a similar manner. For the dimorphism in the spores of genera Nosema, Perezia and Gurleya, one may assume that there occurs dimorphism in the sporont; but in the species of Nosema, I have not seen it if such a state exists. If it occurs in these genera, the dimorphism must be specific. Does the dimorphism of the spore have a significance such as proposed by Vaney and Conte? Guyénot and Naville (1922a) recently expressed their view as to the dimorphism in *Glugea danilewskyi* as follows: "On peut supposer que le premier mode de sporulation appartient à un cycle sexué, le deuxième étant la suite d'un développement asexué ou parthénogénésique." It seems entirely impossible to solve this question in the present state of knowledge. I fail to see any significance in the two modes of sporulation in *Thelohania opacita* and am inclined to think that the so-called dimorphism, in this and other species of Thelohania at least, is only due to the conditions of sporulation and that it does not possess any particular meaning other than abnormality. Why then in some Thelohanias, the nucleus of a sporont divides twice or three times is not clear to me. It is however allowable to assume that conditions related to the nutrition and space in the host cell invaded by the microsporidian may influence the nuclear division.

FORM

The form of the microsporidian spores varies a great deal in different genera as is shown in textfigure A. It is usually oval (2, 3), ovoidal (4-6), ovocylindrical (15) or pyriform (7-11) with the intermediate forms. It may, in some cases, be spherical (1), reniform (12), tubular (16, 17), bacilliform (14), crescent-shaped (13), spiral (18) or comma-shaped (19). As a rule, the spores of one and the same species are somewhat uniform in form, but exceptional cases such as *Mrazekia mrazeki* are also to be met with (Figs. 653,654).

STRUCTURE OF THE SPORE MEMBRANE

The spore is covered by a spore membrane inside of which one finds a sporoplasm and a polar filament which is typically coiled in a polar capsule. The spore membrane or shell is highly refractive and ordinarily of a uniform thickness. Its outer surface is smooth and structureless. In two cases, however, it has been noted that certain markings occurred on the membrane. Thélohan (1895) noticed longitudinal striations on the spore membrane of *Thelohania giardi* (Fig. 403) and Doflein (1898) observed similar markings on that of *Gurleya tetraspora* (Figs. 358-360). In this respect, the spores of Microsporidia differ from those of Myxosporidia in which conspicuous striations are very frequently observed (Kudo, 1920a). Unlike the Myxosporidia, the microsporidian spores rarely bear appendages, only four species being known to possess any. Léger and Hesse (1916) report a caudal prolongation in the spores of *Mrazekia caudata* (Figs. 650, 651), *M. brevicauda* (Fig. 645) and *M. tetraspora* (Léger and Hesse, 1922), the first of which had previously been found by Mrázek (1910). In *Thelohania octospora*, Goodrich (1920) found that each spore possessed three long tails and stated that "the tails are about 20μ long, flattened out proximally but tapering to very fine ends. They show up more clearly when dilute iodine solution is run into the preparation. . . . Iron haematoxylin also stains the tail but is very easily washed out of them with iron alum solutions, and they only show faintly, if at all, after using the ordinary counter stains. Consequently in differentiated films mounted in a refractile medium such as Canada balsam, the tails are practically invisible. Similarly they stain so faintly with Giemsa's mixture, even after many hours that they are again very easily overlooked. These facts account perhaps for their not being described by earlier observers, for there can be no doubt, I think, that the parasite is *Thelohania*



Textfig. A. Typical microsporidian spores showing differences in dimension and form. 1, *Cocconema microccus* after Léger and Hesse; 2, *Thelohania rotunda* after Kudo; 3, *Nosema bombycis* after Kudo; 4, *Glugea anomala* after Weissenberg; 5, *Telomyxa glugeiformis* after Léger and Hesse; 6, *Plistophora macrospora* after Léger and Hesse; 7, *Glugea acuta* after Thélohan; 8, *Nosema cyclopis* after Kudo; 9, *Thelohania giardi* after Mercier; 10, *Gurleya francottei* after Léger and Duboscq; 11, *Stempellia magna* after Kudo; 12, *Thelohania reniformis* after Kudo and Hetherington; 13, *Octosporea muscae-domesticae* after Flu; 14, *Mrazekia bacilliformis* after Léger and Hesse; 15, *Nosema marionis* after Thélohan; 16, *Mrazekia mrazeki* after Hesse; 17, *M. argoisi* after Léger and Hesse; 18, *Spironema octospora* after Léger and Hesse and 19, *Toxonema vibrio* after Léger and Hesse. The scale is given in microns.

octospora (See Fig. 443 in this paper)." Because of the minuteness of the average spores, it is a matter of dispute whether the spore membrane is composed of two valves as in myxosporidian spores or is a single piece. The majority of observers have agreed in maintaining that the latter is the case. This view is held even by those who believed they have noticed two parietal nuclei which controlled the formation of the spore membrane.

On the other hand, there have been some authors who actually observed a longitudinal line on the spore membrane which they interpreted as a sutural line of two shell-valves. Thélohan (1895) observed such a line on the spores of *Glugea anomala* (Fig. 252) and more distinctly in *Thelohania giardi*, which he held as a sutural line. Lutz and Splendore (1904) figure a spore of *Plistophora simulii* in which the two shell-valves split after the extrusion of the polar filament (Fig. 608). Mercier (1908a) noticed the sutural line on the spore of *Plistophora* sp (Fig. 636). In a small spore of *Nosema apis*, Fantham and Porter (1912a) figures a small spore "showing line of weakness." In *Thelohania opacita*, I have noted that the polar filament was extruded under mechanical pressure either from the side or from the end of the spore and that in moderately compressed spores there was a longitudinal line distinctly visible on the spore membrane (Figs. 570, 750), and maintained that some spores of this species possess a bivalve membrane. Stempell (1902, 1904, 1909) considered the spore membrane of *T. mülleri*, *Glugea anomala* and *Nosema bombycis* was a single piece, although it developed from two parietal cells during the sporulation. Schuberg's (1910) observations on *Plistophora longifilis* led him to consider that there were no parietal nuclei reported by Stempell, Mercier and others and that the membrane was a single piece. Debaisieux (1919, 1919a, 1920) and Guyénot and Naville (1922a) agreed with Schuberg on this point.

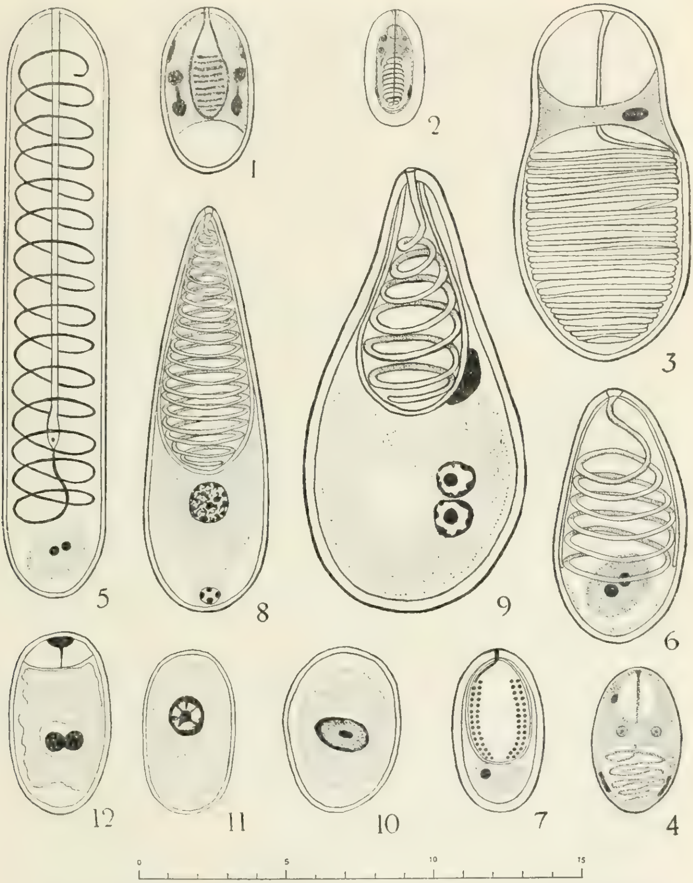
In nearly all the species of Microsporidia which have come under my observation, I have regularly found that the spore membrane was a single piece, but in the case of *Thelohania opacita* as was stated above, the membrane seemed to be composed of two valves (Fig. 570). From this one may conclude that the spore membrane of microsporidian spores is in many cases a single piece, while in some cases, it is bivalve, which is one of the points of close resemblance between Microsporidia and Myxosporidia. Little is known concerning the chemical nature of the structure. Nägeli saw no blue staining of the spore membrane of *Nosema bombycis* by iodine and sulphuric acid treatment. Frey and Lebert (1856) and Haberlandt and Verson (1870) showed that the spores of *N. bombycis* are highly resistant against certain chemicals. Thélohan (1895) held that the shell was not of cellulose nature. After a series of experiments, I came to the conclusion that "the spore membrane of *N. bombycis* and *N. apis* behaves very much like chitin under the influence of mineral acids" (Kudo, 1921c, April). Koehler (1921, July) came to a similar conclusion stating that "die Sporenschale der *N. apis* aus chitin besteht."

THE INTERNAL STRUCTURE OF THE SPORE

In the fresh state, the microsporidian spore is characterized by its strong refractivity—a unique appearance—due to its spore membrane. The refractivity of the spore membrane shows some variation according to its thickness and in many cases like that of Canada balsam, an ordinary mounting medium, the spore appears to be smaller in the medium than in fresh preparations. It should, of course, be remembered that the decrease in dimensions of the spore is frequently due to fixation and subsequent treatment with dehydrating reagents. The spores of many species show a conspicuous vacuole or clear space at one end, which is ordinarily more rounded than the other. This vacuole may be seen in all the spores of a species such as in *Nosema cyclopis*, *N. infirmum*, *Thelohania opacita*, *T. pyriformis*, etc., or may be seen only in a certain number of spores, in which case young spores seem to show the vacuole, while the older ones do not. In general when the spore membrane is thin the vacuole is regularly observable. The part of the spore unoccupied by the vacuole may either be finely granulated or show transverse strands. Contrary to the myxosporidian spore, the nucleus is not noticed in fresh state.

As to the finer structure of the microsporidian spore, great diversity of opinion prevails. This is doubtlessly due to the smallness of the object, to the peculiar nature of the spore membrane which obscures the internal structure and to the dissimilarity in structure in different species. Unless optical apparata and microscopic technique are improved, the controversy regarding the structure of the microsporidian spores will continue.

Thélohan (1895) stated that the spores are refringent and have a large vacuole at one end. Upon treating with nitric acid (66 per cent heated to 36° C) a pyriform polar capsule becomes visible near the other extremity. Normally the capsule is surrounded by a coat of protoplasm and is invisible. In the sporoplasm three nuclei are noticed. Two nuclei are for the sporoplasm proper and the other is for the polar capsule in which the filament is coiled. Stempell (1904) first gave a schematic figure (Fig. 262) of a spore of *Glugea anomala*. According to his view, the spore has the following structures: Inside the shell, one small and the other large vacuole are located at the anterior and posterior end respectively. The sporoplasm is girdle-like in form and is located between the vacuoles. It contains four nuclei when fully prepared to germinate in the gut of a new host. The anterior vacuole seems to have been thought to be a polar capsule, although Stempell showed that the polar filament beginning at the side near the anterior tip, was coiled back through the protoplasm to the posterior vacuole, the larger part of the filament being located in the posterior vacuole. Léger and Hesse (1907) stated briefly that the spore of *Nosema bombycis* has a structure similar to that of *Coccomyxa morovi*, i. e.,



Textfig. B. Structure of the spores of Microsporidia, Myxosporidia and Haplosporidia. 1, *Thelohania giardi* after Mercier; 2, *Nosema bombycis* after Stempell; 3, *Plistophora longifilis* after Schuberg; 4, *Nosema apis* after Fantham and Porter; 5, *Mrazekia argoisi* after Léger and Hesse; 6, *Plistophora macrospora* after Léger and Hesse; 7, *Nosema apis* after Kudo; 8, *Stempellia magna* after Kudo; 9, A myxosporidian, *Myxobolus toyamai* after Kudo; 10-12, Haplosporidian spores after Swellengrebel, Perrin and Swarczewsky respectively. The scale is given in microns.

the spore showed the valve-cells, a capsulogenous cell with a very minute nucleus and the sporoplasm with one large or two smaller nuclei.

In his remarkable study on *Thelohania giardi*, Mercier (1908, 1909) observed that the shell develops from two valve cells, that the initial phase of the polar capsule is a vacuole appearing inside the sporoblast (Fig. 431), which develops into a pyriform sac with a coiled filament, occupying about two-thirds of the intrasporal space with its end attached to the narrow extremity, that the sporoplasm takes a girdle-like form at the central part of the spore, leaving a vacuole at each end and that the sporoplasm possesses two and later four nuclei (Textfig. B, 1). Schröder (1909) in his study of *T. chaetogastris* expressed a similar view to that of Mercier. He however mentioned that the sporoplasm had two nuclei or one, the latter being formed by a fusion of the two. In 1909 Stempell published his work on *Nosema bombycis* and gave a schematic representation of a spore (2). On the whole, he confirmed Mercier's view mentioned above. Contrary to his previous observations, Stempell now recognized the presence of a polar capsule and maintained that the greater portion of the filament is coiled in the posterior vacuole around a central axis which runs longitudinally in the spore beginning at the anterior end. After studying the spores of *Plistophora longifilis*, Schuberg (1910) came to the conclusion that the girdle-like sporoplasm which is a ring in cross-section, contains only a single nucleus, that the polar filament which reaches 510μ in length is coiled directly under the shell mostly in the posterior portion of the intrasporal space, that the so-called polar capsule does not occur in the spore, and that the nuclei observed by other observers are none other than the volutins or metachromatic granules which appear in the spore by fixation and staining as artefacts (3).

Fantham and Porter (1912a, 1914) maintained the structure of the spores of *Nosema apis* and *N. bombi* was as follows (4): They distinguished two nuclei in the sporoplasm, two for the shell and one for the polar capsule which is identical with the small vacuole judging from the figure they gave. The polar filament passes as a straight rod backward through the polar capsule, and after passing through the sporoplasm, it becomes spirally coiled in the posterior vacuole. Brug (1914) in his study on *Octosporea monospora*, stated that the granules referred to by Schuberg are at a certain stage connected with the nuclei by threads, that they arise from nuclei, but that whether they were really nuclei could not be determined. Weissenberg (1913) observed that the spores of *Glugea anomala* and *G. hertwigi* were similar to that of *Plistophora longifilis* and showed a rather large rounded volutin grain in the larger vacuole (Figs. 325, 328-330).

In the spore of *Nosema bombycis*, I (Kudo, 1916) recognized structures (Figs. 32, 33) similar to those observed by Mercier and Stempell except that the sporoplasm contained two nuclei, that there is a thin protoplasmic membrane directly inside the spore membrane which was also observed by Ishiwata in *Nosema* sp., that the polar filament was much longer than it was thought to be and that the filament was probably coiled as in Myxosporidia (Textfig. B, 9). Léger and Hesse (1916) described under the generic name of Mrazekia an interesting group of Microsporidia which are characterized by cylindrical or tubular spores (5). The polar filament is composed of two parts: a proximal rather solid part which runs along the axis of the spore and a more slender distal portion which is coiled around the former. No polar capsule is mentioned. The binucleated sporoplasm is a rounded and more or less well defined body imbedded in a clear space at the posterior end of the spore.

The same authors (1916a) further studied the spores of *Plistophora macrospora* (6) and found that the polar capsule lies close to the shell, occupying the greater part of the intrasporal space, and the filament is spirally coiled six times around the central axis inside the capsule. The sporoplasm was observed as a rounded binucleated body embedded in the posterior vacuole. This structure was noticed in the spores stained by the silver impregnation method (Fig. 614) or picro-carmin (Fig. 616). In the spores fixed with osmic acid and stained with iron hematoxylin, structures similar to those observed by other authors were found (Fig. 615). Thus in the center, one sees a girdle-like mass in which deeply staining granules are found, which are in reality contracted substances composing the capsule; a part of the filament is visible as a short thread at the anterior end; a deeply staining granule seen in the posterior vacuole, which is not a metachromatic granule as was thought by Schuberg and others but the true nucleus of the sporoplasm, and the deeply staining granules found in the girdle-shaped mass are none other than one or two turns of the polar filament viewed in optical section.

Georgévitch (1917) in his brief study of *Nosema marionis* agreed with Léger and Hesse's view (Figs. 68, 69) quoted above, but added that he also saw that the polar capsule was entirely unobservable in some spores (Fig. 71). Paillot (1918) recognized the binucleated sporoplasm in the spore of *Perezia mesnili* in which the filament was coiled in a way (Fig. 344) similar to that observed in *Nosema apis* by Fantham and Porter (Textfig. B, 4). Debaisieux (1919) after studying carefully the spores of *Thelohania varians* came to agree with Schuberg as to their structure.

My recent study on this subject has led me to make the following statements. In *Stempellia magna* (Kudo, 1920, 1921a, 1922, 1924b) a conspicuous polar capsule occupies the anterior two thirds, while in the remaining part of the spore is found a binucleated sporoplasm (Textfig. B, 8). In *Nosema apis* (Kudo, 1921), a similar structure was noticed. But the sporoplasm seemed to be uninucleated and the polar filament "is coiled from 10 to 15 times along the polar capsule, inside of which and continuous to it, it is coiled back again toward the tip where the filament is attached (7; Fig. 148). Guyénot and Naville (1922a) observed in the spore of *Glugea danilewskyi* that the sporoplasm is binucleated and the polar capsule with its coiled filament is located at the other extremity (Fig. 295).

From the statements quoted here it is clear that the microsporidian spore is composed of three parts in all the well studied forms; i. e., the spore membrane, a sporoplasm and a polar filament, and that there are diverse opinions as to the finer structure of the sporoplasm such as its location and nucleus and the presence of a polar capsule. Even in one and the same species of Microsporidia, observations of different authors differ considerably one from the other. For instance, Debaisieux (1919a) and Guyénot and Naville (1922a) both working on apparently one species, *Glugea danilewskyi*, and presumably using up-to-date optical apparatus and technique, came to entirely different conclusions as to the structure of the spore. The same is true in the spore of *Nosema apis* as studied by Fantham and Porter (1912a; see Textfig. B, 4) and by Kudo (1921; 7). It is to be regretted that the majority of the authors studied spores that were less than 6μ in the largest dimensions which naturally made the observations very uncertain. Only in a few cases were authors fortunate enough to study spores larger than 10μ in length under favorable conditions. Among them one may mention Schuberg (*Plistophora longifilis*), Kudo (*Stempellia magna*) and Léger and Hesse (Mrazekia and *Plistophora macrospora*); yet here one finds diversities of observations as in other smaller forms.

One is led to believe that the microsporidian spores, as I have already mentioned elsewhere (Kudo, 1921a), are not unique in their structure. It is interesting to note here that the structural variation in different microsporidian spores seems to be limited by Myxosporidia on one hand and Haplosporidia on the other hand. In the unicapsulated Myxobolus (9) one finds the pyriform spore containing a polar capsule with a coiled polar filament and a binucleated sporoplasm, which is exactly similar to the spores of *Plistophora macrospora*, *Stempellia magna*, etc. In *Telomyxa glugeiformis*, the only bicapsulated microsporidian, one finds a close relationship between it and Myxosporidia belonging to genera Myxidium and Zschokkella (Kudo, 1920a). On the other hand forms such as *Octosporea muscae-domesticae* or *O. monospora*, seem to show a very close affinity to Haplosporidia (Textfig. B, 10 to 12). Between the two extremes are to be found intermediate forms which form the bulk of the Microsporidia.

THE POLAR FILAMENT

The polar filament is a characteristic structure of the microsporidian spore. It is conspicuous in that it is extremely fine and long—in *Plistophora longifilis*, it measures 380 to 450μ or even up to 510μ, in *Nosema apis* 230 to 280μ long (Kudo) and up to 400μ (Morgenthaler) and in *Thelohania fibrata* 170 to 220μ long. The filament was first discovered by Thélohan (1892) in *Glugea anomala* (Fig. 255). Thélohan (1895) stated that it is of variable length and extreme fineness. Whether the filament is a solid thread or hollow tube is undetermined at present due to its minuteness. Stempell (1909) gives a hypothesis for that of *Nosema bombycis* as follows: "Um wie minutiöse Bildungen es sich hier handelt, wird besonders deutlich, wenn man bedenkt, dass dieser höchstens 0.1μ dicke Polfaden in Wahrheit ja eine Röhre ist, deren Wände also selbst wenn man das Lumen gleich O setzt, höchstens eine Dicke von 0.05μ besitzen können!" For *Thelohania fibrata*, Strickland (1913) agreed with Stempell stating that "it should be borne in mind, as Stempell points out, this filament must, from the manner in which it is ejected, consist of a hollow tube which has to be entirely everted during its emergence from the spore!" It is of course beyond the limit of direct observation.

The polar filament is uniformly fine throughout. In the genus Mrazekia, however, the filament is composed of two portions: the more or less thick basal rod-like structure ("manubrium") and the other uniformly fine filament (Textfig. B, 5; Figs. 643, 644). In other species, the end with which the filament is attached to the spore when extruded, is usually thickened in a round form. The free end of the filament tapers to a point in most cases. When the filament is not completely extruded under artificial conditions, one may notice a small knob at the free end of the structure. Ishiwata (1917) saw numerous filaments with rounded ends in *Nosema* sp. (Figs. 184, 185) and suggested that the filament is probably adhesive. Morgenthaler (1922) observed in some filaments of *N. apis* "eine flüssige Masse, manchmal in einem recht kräftigen Guss, aus ihrem Ende austreten, die sich dann um die Spitze des Fadens herumlagert." I have noticed a similar condition in some of the filaments of the species which came under my observation. In *Stempellia magna* (1924b) I found that very rarely one sees a thick point at the extremity of the extruded filament, examination under a higher magnification shows, however, that here the filament probably became broken during extrusion and the material which composed the filament became spread out as a result (Figs. 593-596).

As to the manner with which the polar filament is coiled within the polar capsule the following are on record.

1) The filament is coiled in a way similar to that of *Myxobolus toyamai* (Textfig. B, 9) as was seen in *Plistophora macrospora*, *Stempellia magna*, etc.

2) In *Nosema apis*, I have noted the peculiar situation of the filament and stated that "the filament is doubly coiled" (Textfig. B, 7; Fig. 148).

3) The basal portion of the filament runs longitudinally along the axis and the remaining part is coiled back on the former as is observed in Mrazekia (Textfig. B, 5).

4) The filament is sometimes coiled longitudinally as was seen in *Nosema* sp. by Ishiwata (Figs. 185, 186).

The extrusion of the filament in the spores of *Nosema bombycis*, *N. apis*, *N. bombi*, *Thelohania mülleri* and *Stempellia magna* was seen to take place experimentally in the digestive fluid of the hosts. Aside from this, it occurred when the spores were brought under the influence of the following reagents or circumstances.

Acetic acid: *Nosema apis*, *N. bombi*, *N. ctenocephali*, *Thelohania mülleri.*

Ammonia water: *Plistophora longifilis.*

Ether: *Thelohania octospora.*

Glycerine: *Nosema bombi*, *Glugea anomala*, *Thelohania pinguis*, *T. ovicola.*

Hydrochloric acid: *Glugea danilewskyi*, *Thelohania varians*, *T. giardi.*

Iodine water: *Nosema apis*, *N. bombi*, *N. ctenocephali*, *Glugea anomala*, *G. acuta*, *Thelohania mülleri*, *T. janus*, *T. legeri*, *T. cepedei*, *T. varians*, *Plistophora typicalis*, *P. mirandellae*, *P. acerinae*, *P. vayssierei.*

Mechanical pressure: *Nosema bombycis*, *N. apis*, *N.* sp. Ishiwata, *N. baetis*, *N. cyclopis*, *N. infirmum*, *N. anophelis*, *Thelohania mülleri*, *T. legeri*, *T. opacita*, *T. mutabilis*, *T. baetica*, *T. obesa*, *T. pyriformis*, *Stempellia magna.*

Nitric acid: *Nosema bombycis*, *N. bryozoides*, *N. bombi*, *Glugea cordis*, *Thelohania giardi*, *T. mülleri*, *Plistophora* sp. Mercier.

Perhydrol: *Nosema bombycis*, *N. apis.*

Physiological solution: *Gurleya richardi*, *Plistophora vayssierei*, *P. macrospora*, *Mrazekia mrazeki.*

Sulphuric acid: *Gurleya legeri*, *Plistophora vayssierei.*

Water, distilled: *Nosema apis.*

The foramen through which the filament is ejected is usually at the end which is ordinarily slightly attenuated. In some spores this seems to be at the tip, while in others not at the tip. At the time of extrusion, the filament is not straight, but shows numerous windings (Fig. 148). These windings possibly indicate the number of turns of the filament while coiled inside the spore. Sooner or later, however, it becomes straightened. Under the action of reagents such as perhydrol, the polar filament is seen to shoot out with great rapidity and the phenomenon takes place immediately and lasts for several minutes (Kudo, 1918). On the other hand one sees frequently rather slow shooting under the cover-glass due probably to a light pressure of the cover glass (Kudo, 1924a). In either case, the spore exhibits a vigorous vibration at the moment of filament extrusion. Many filaments become detached completely from the spore while under the cover glass.

Concerning the mechanism of filament extrusion in *Nosema bombycis*, Stempell (1909) stated that "bei der Neuinfektion gelangen sie dann—oft nach einer längeren oder kürzeren Trockenperiode—in die Darmflüssigkeit einer Raupe, die jedenfalls ärmer an Salzen ist, als die Gewebeflüssigkeit, und es wird daher, falls die Sporenhülle eine halbdurchlässige Membran darstellt, durch Osmose Wasser in die Spore eintreten, und in dieser unter gleichzeitiger Quellung des Protoplasmas ein Druck entstehen, der dann den Polfaden zur Ausstülpung bringt."

After studying the action of perhydrol upon the spores of the same species, I noticed (Kudo, 1918) that "it seems probable that a physical force, comparable to that produced by pressure, develops within the spore and expels the polar filament. This force may be none other than the gas evolved through the decomposition of hydrogen peroxide by the peroxydase contained within the spores. In support of this view it may be cited that the spores preserved for more than one year still extruded their filaments when subjected to mechanical pressure. The failure of the dried spores, therefore, to extrude the polar filament under the action of perhydrol is a sign of the weakening or absence of the peroxydase, but bears no relation to the viability of such spores."

Morgenthaler (1922) who caused filament extrusion in *Nosema apis* in a hanging drop preparation with distilled water stated that "wenn aber reines Wasser die Ausstülpung der Polfäden bewirken kann, so geht man wohl nicht fehl, wenn man in dem ganzen Vorgang eine Quellungserscheinung sieht. Man müsste dann einen quellbaren Körper in der Polkapsel annehmen, welcher bei Berührung mit Wasser aufquillt und den Polfaden hinausstösst."

With reference to the function of the polar filament, very little is known at the present time. Since the noted dispute between Balbiani and Leuckart and Bütschli, numerous authors have contributed opinions as to the significance of the polar filament of myxosporidian spores, yet very few discussions were concerned with Microsporidia. Since Leuckart (1879) suggested that the polar filament of Myxosporidia is an attachment apparatus, Bütschli (1882), Thélohan (1895), Doflein (1899) and others have expressed similar views. This view was carried over to microsporidian spores. For the filament of *Nosema bombycis*, Stempell (1909) makes the following statement: "Die Aufgabe des ganzen Polfadenapparates besteht vielleicht darin, die Spore am Epithel des Darmes zu verankern, damit sie nicht mit dem Strom des Darminhaltes fortgeschwemmt wird. In der Tat glaube ich in einem einzigen, günstigen Fall gesehen zu haben, wie ein allerdings schon von der Spore losgelöster Polfaden an Epithel hing." Fantham and Porter (1912) after observing *N. apis*, wrote that "occasionally, as at times of rapid induced currents in the bee's gut, the spores thrust out their polar filaments, which hook into the cement between the epithelial cells and temporarily anchor the parasite." Korke (1916) in his study on *N. ctenocephali* states that "the function of the filament has been stated to be the fixation of the spore to the gut epithelial cell; but it may be that it also serves to conduct the sporozoite to a distant part of the tissue and thus ensure an advance of the parasite into fresh areas" (Figs. 181, 182).

At present no definite information can be given about the exact chemical nature of the polar filament of cnidosporidian spores. Thélohan (1895) stated that the substance composing the wall of the polar capsule was identical with that which makes up the spore membrane since both stained in the same way with safranin. This probably led some authors to state without proof that the polar filament of some cnidosporidian spores is chitinoid in nature (Minchin, 1912). I have carried out a few experimemts which show that the polar filaments of cnidosporidian spores are not composed of glycogen as was suggested by Erdmann (1917) and that the filaments are formed by a mixture of a part of the nucleus and a substance differentiated in the capsulogenous cell (Kudo, 1921c).

THE VEGETATIVE FORM

None of the Microsporidia have hitherto been cultivated on artificial media. For this reason, the study of the development has almost exclusively been made with material obtained through experimental infections. When introduced into the digestive tract of the host, the microsporidian spores sooner or later extrude their filaments under the influence of the host's digestive fluid. As was discussed elsewhere the polar filament serves for attachment of the spore to the gut epithelium when its distal end comes in contact with the latter. This would naturally bring the spore closer to the host epithelial cells. The filament becomes detached from the spore and leaves a small foramen in the spore membrane. It is through this foramen that the sporoplasm creeps out as an amoebula. This change has been noted in *Nosema bombycis* (Stempell 1909, Kudo 1916), *N. apis* (Fantham and Porter, 1912a), *N. bombi* (Fantham and Porter 1914), *Thelohania müllеri* (Stempell 1902) and *Stempellia magna* (Kudo, Fig. 571).

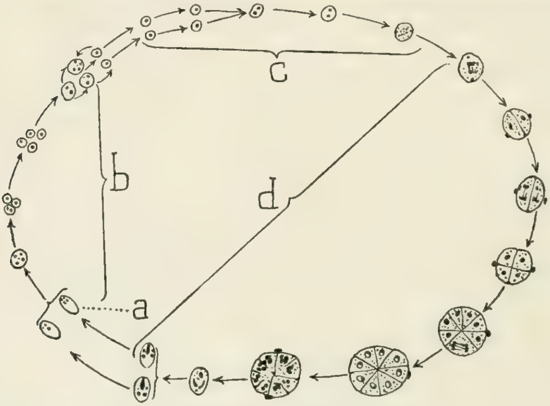
Pfeiffer (1888) writes that in hanging drop preparations he saw the emergence of the sporoplasm from the spores of *Nosema bombycis*, although his figures do not convince one that he had seen the sporoplasm. Sasaki (1897) observed that when the spores of *N. bombycis* were studied in the blood of the silk-worms, the sporoplasm emerged in the form of a small amoebula either from one of the poles or from the side. In *Perezia legeri*, Paillot noticed that the filament was ejected from one end, but in at least one case he figured a spore whose sporoplasm was escaping from the side of the spore (Fig. 354).

After treating the spores with dilute hydrochloric acid, Guyénot and Naville (1922a) saw the rupture of the spore membrane of *Glugea danilewskyi* close to the end to which the extruded filament was attached and supposed that the sporoplasm escaped through this opening (Fig. 294). In the case where the spore membrane is composed of two shell valves, extrusion is probably followed by the separation of the two valves and through this opening the sporoplasm leaves the spore as an amoebula, as was noted in *Plistophora simulii* (Lutz and Splendore, Fig. 608) and *Thelohania opacita* (Kudo, Figs. 570, 750). The entire process of emergence of a living amoebula from a spore has unfortunately not been observed in any case. It seems to be fairly certain, however, that the emergence of the sporoplasm takes place in the lumen of the gut of the host. Judging from the irregular shape of the amoebulae found in stained preparations, several authors agree in assuming that these young stages are capable of amoeboid movements. Fantham and Porter (1912a) write that in *Nosema apis* the sporoplasm, retaining two of the nuclei, creeps out from the sporocyst, leaving the two sporocyst nuclei behind. Then the free sporoplasm becomes amoeboid, and the binucleate amoebula creeps about over the intestinal surface, although they do not give any figures to clearly show this interesting observation. The changes that take place between this stage and the intracellular stages are not clearly known. Balbiani (1884) maintained that the amoebulae penetrated through the wall of the epithelial cells and begun interacellular development. The conception which some authors accept at present is founded on what Stempell (1909) described for *Nosema bombycis*. According to this author the binucleated amoebula (Textfig. D, w) transforms itself into planonts (a, b, c) which multiply actively by binary fission or budding, and which by amoeboid movements swim around in the lumen of the alimentary canal and in the intercellular spaces of the host body. After leading the extracellular life in the haemocoel, the binucleated planonts enter the host cells and begin intracellular development (d). Kudo (1916) could not confirm this planont stage of the same parasite. Stempell's view seems to fit for an interpretation of the fact that the microsporidian invades different tissue cells of the host silkworm in a comparatively short time. Zander (1911) and Fantham and Porter (1912) in *N. apis*, further Fantham and Porter (1914) in *N. bombi* and Korke (1916) in *N. ctenocephali* recognized a similar stage, adopting also the term planonts.

The larger part of the multiplication and sporulation periods of the Microsporidia takes place in the host cells. The early intracellular stages are ordinarily called schizonts. Some authors, however, follow Stempell by calling them meronts, a term coined by the latter author (1902) for *Thelohania mülleri*. The development and divisions of the schizonts leading up to the formation of the sporonts which produce sporoblasts and further spores, are collectively called schizogony, while the changes between the sporont and the spore are ordinarily included in sporogony.

The schizonts are more or less rounded bodies of various dimensions. They typically possess one nucleus. They are incapable of movement. The cytoplasm takes stain more deeply than that of the sporont. The schizont grows at the expense of the cytoplasm of the host cell in which it is lodged. The nutrition is taken in by osmosis. In many instances there is a narrow but clear space around each schizont. Stempell (1909) noted this condition in the schizont of *N. bombycis* and explained that it is capable of secreting a peptonizing ferment and liquifies the host cytoplasm around it, which is absorbed by osmosis.

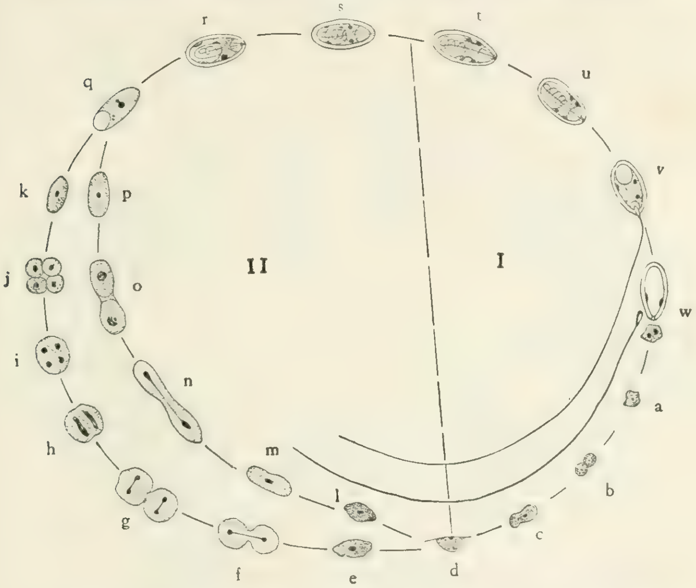
When the growth reaches a certain point, the schizont undergoes multiplication. The division most commonly seen is a binary fission. The nucleus divides into two daughter nuclei, each moving toward the opposite pole. The cytoplasm becomes constricted in the middle and two daughter schizonts are produced. Occasionally one finds unequal binary division which is sometimes referred to as budding. Under certain conditions and in some species, the division of the nucleus is not directly followed by complete separation of the cytoplasm. The nuclei divide further producing various chain or sausage forms. Multiple division sometimes occurs in some species.



Textfig. C. The life cycle of *Thelohania giardi*. After Mercier.

At the end of schizogony, the sporonts are formed. In the genus Nosema a single sporont transforms itself into a single spore. In other genera, the sporont grows and its nucleus divides into 2, 4, 8, 16 or many daughter nuclei, each of which becomes the nucleus of a sporoblast and thus the pansporoblasts with 2, 4, 8, 16 or many sporoblasts are produced. Each sporoblast develops into a spore.

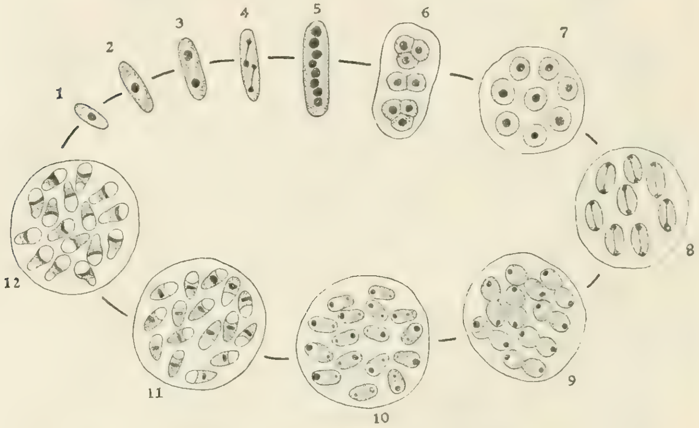
The first life cyle of a microsporidian was given by Mercier (1908, 1909) for *Thelohania giardi* in which he had the advantage of seeing distinctly the stages of schizogony and sporogony. In the digestive tract of *Crangon vulgaris* the spores germinate (Textfig. C, a) and the amoebulae enter the body cavity where the parasite multiplies very actively by simple or multiple schizogonic division (b). The schizonts then invade the muscular tissue and penetrate the muscle fibers where they continue to multiply. The end of schizogony is characterized by the appearance of small uninucleated bodies which become coupled two by two. This is followed by karyogamy (c). Thus the sporont is formed by isogamy. During this process, chromidial apparatus appears which becomes collected in two masses and controls the development of the sporont into a pansporoblast. The nucleus of the sporont divides three times and produces eight sporoblast nuclei. Each sporoblast is transformed into a spore in the following way: the nucleus divides and produces two valve nuclei, a capsulogenous nucleus and two sporoplasm nuclei (d). The spores become liberated from the host



Textfig. D. The life cycle of *Nosema bombycis*. After Stempell. I extracellular stages: II intracellular stages. a-c, planonts; d-p, meronts; q-s, stages in sporulation; t, u, spores in the mid-gut of a new host; v, extrusion of the polar filament; w, amoebula leaving the spore.

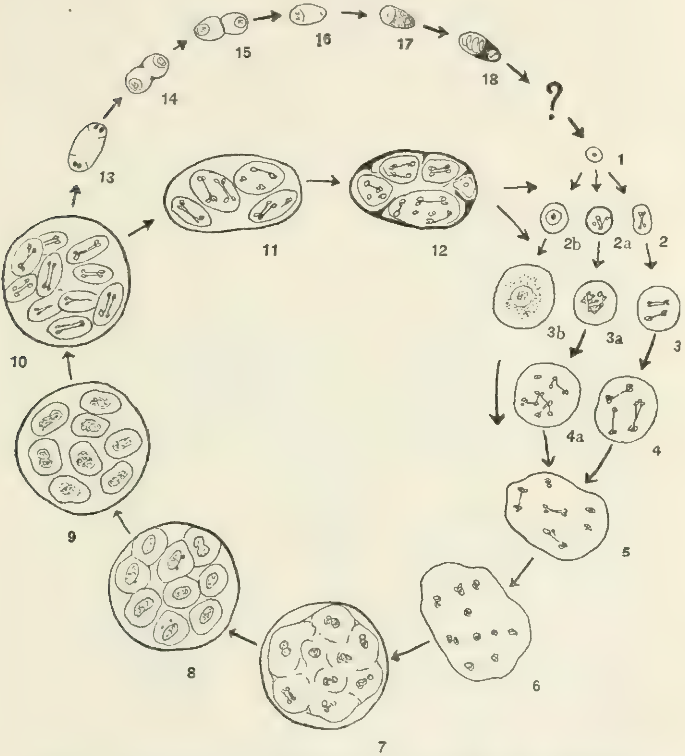
by accidental rupture of the infected tissue or by death of the host. The nuclei of the sporoplasm later divide into four.

For *Nosema bombycis*, Stempell (1909) gives the following life cycle. The earliest stage is a small uninucleated amoeboid body (the planont) which appears in numbers particularly in the blood stream of the host larvae soon after the infection (Textfig. D, a). These planonts multiply by a binary fission (b), become distributed throughout the entire host body, enter different tissue-cells of the host and become meronts (c, 1). These meronts are spherical or oval in shape and divide actively by fission (m to p), budding or multiple division (f to k). The host cell finally becomes completely filled with schizonts. When the nutrition becomes exhausted and the space too small, each meront develops into a spore. The meront becomes elongated ovoid and its nucleus divides, forming two shell-nuclei, a nucleus for the polar capsule and two sporoplasm-nuclei. The spore membrane is formed and vacuoles appear at the poles. The sporoplasm assumes a girdle-shape in the middle of the spore and the polar capsule with its coiled filament becomes located along the axis of the spore (q to s). After the host cells disintegrate, the spores become liberated and leave the host body. When taken into the digestive tract of another host larva with the food, the two nuclei of the spore divide once producing four nuclei of equal size (t, u). The polar filament is extruded and later becomes detached. Through the foramen thus made the binucleated sporoplasm creeps out,



Textfig. E. Development of *Glugea anomala*. After Weissenberg.

while the other two nuclei degenerate in the spore. The two nuclei in the amoebula fuse into one and the planont is formed. Under favorable conditions, the entire life cycle is completed in four days.



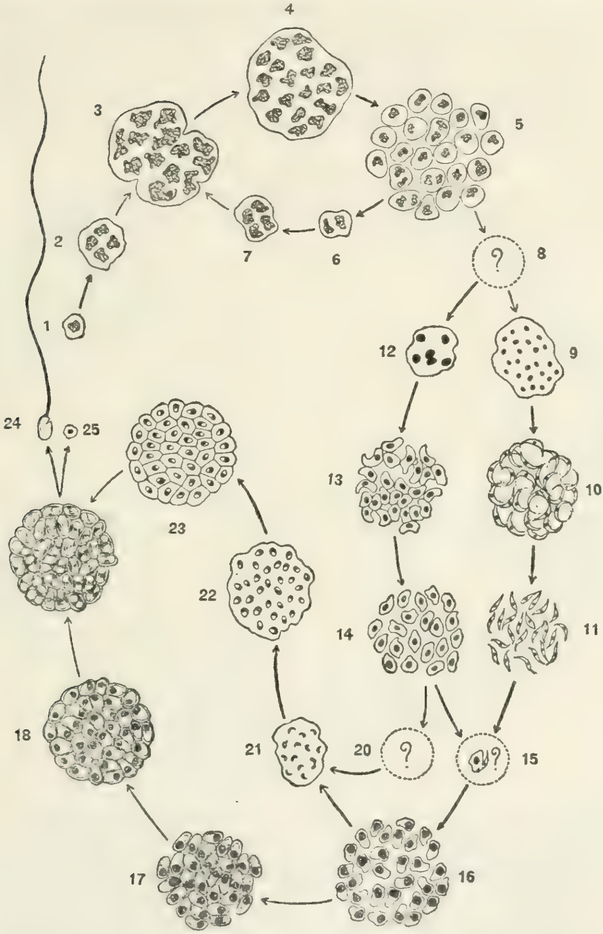
Textfig. F. The life cycle of *Glugea danilewskyi* and *G. mülleri*. After Debaisieux.

According to Weissenberg, the following are the main features in the development of *Glugea anomala*. The infection of a new host individual takes place through the mouth and the germination of the spore apparently in the gut. Young uninucleate schizonts invade host cells (Textfig. E). They grow larger and undergo a binary fission (2, 3). In some schizonts the nucleus divides repeatedly without being accompanied by cytoplasmic constrictions. Thus cylindrical bodies with eight compact nuclei are formed (4, 5). A vacuole becomes differentiated around these schizonts which divide into eight uninucleate bodies or "vacuole cells" (6, 7). These are apparently sporonts (7). Each sporont divides into two sporoblasts (8, 9) which in turn develop into two spores (10 to 12). The host cell becomes enormously hypertrophied during these changes and assumes conspicuous dimensions, forming the so-called glugeacyst. Debaisieux (1920) found in *Glugea anomala*, that the sporont or the zygote is formed by an autogamy as in the case of *G. danilewskyi* and *G. mülleri*.

Debaisieux (1919a) gives the following life cycle (Textfig. F) for the two species last mentioned. The youngest stage in a newly infected host is uninucleated bodies (Textfig. F, 1) which develop into plasmodia by nuclear divisions and growth (1 to 5). In *G. danilewskyi* often a simultaneous dispersion of the daughter nuclei takes place (2a to 4a), while in *G. mülleri* a large nucleus is often formed (2b, 3b). The plasmodium (5, 6) divides into autogamous copulae (7 to 9). These are the zygotes or the sporonts. Each divides into two sporoblasts (10, 13 to 15) and further develops into two spores (16 to 18). Another cycle which takes place in a host body starts as in the other with a vegetative multiplication (1 to 5) which is followed by formation of autogamous copulae as in the other cycle (6 to 9). These go back to uninucleated or plasmodial vegetative stages without transforming themselves into sporoblasts (11, 12, 1). The so-called cyst membrane is a part of the infected host cell and not of the parasite.

In contrast with Debaisieux's view, Guyénot and Naville (1922a) report the following life cycle for *Glugea danilewskyi*. In the intramuscular cysts, the schizogony is represented by large multinucleated amoeboid bodies which divide by plasmotomy and which undergo multiple division to form uninucleated amoebulae. These latter bodies can repeat the vegetative multiplication (Textfig. G, 1 to 7). The sporulation seems to be preceded by gametogenesis which results in the formation of rounded macrogametes and falcated microgametes. The copulation was however not seen (8 to 15). There are two types of sporulation. a) Macronucleate type: the sporoblasts are independent from one another and possess a large nucleus and deeply staining cytoplasm. They develop into macrospores (16 to 19). b) Micronucleate type: the sporoblasts remain in a common mass. The cytoplasm stains feebly and the nuclei are very small. They give rise to microspores (21 to 23). The authors consider that the first type of the sporulation is a sexual cycle and the second is the result of asexual or parthenogenetic development.

In the life history study of a microsporidian based upon fresh preparations and fixed smears and sections, one encounters many difficulties,



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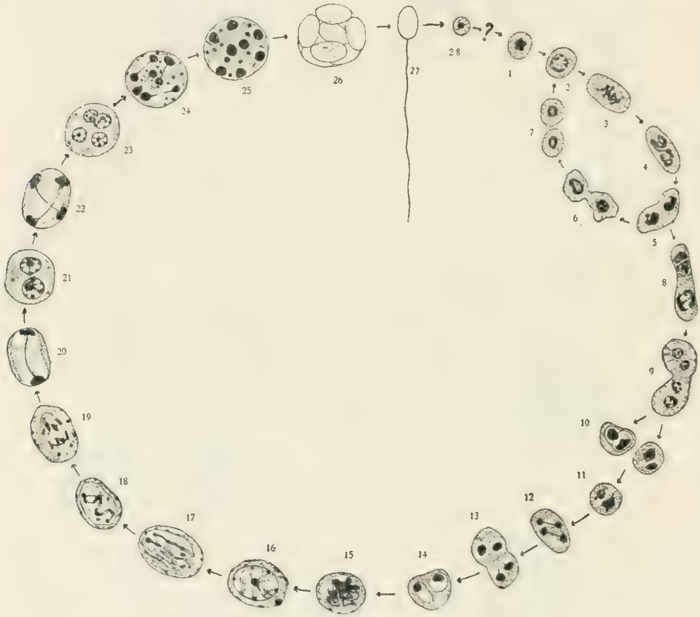
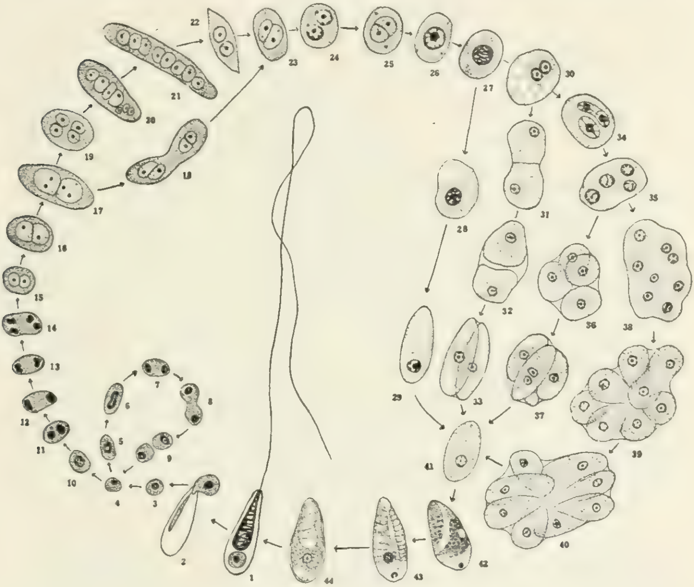
Textfig. G. A scheme of the life cycle of *Glugea danilewskyi*. After Guyénot and Naville.

particularly in forms belonging to the genera Nosema, Glugea, Perezia, etc., because of the fact that the stages in sporogony possess appearances more or less similar to those in schizogony. On the other hand in the species of the genus Thelohania, the sporogonic stages are sharply distinguished from other stages. I was fortunately able to study the life history of *Thelohania legeri*, a typical microsporidian parasite of several species of anopheline mosquitoes (Kudo, 1921a, 1922, 1924, 1924a) which is as follows: The youngest stages found in the infected fat body cells of the host are rounded bodies with compact chromatin granules (Textfig. H, 1). The earlier stages are unknown. The nucleus becomes vesicular and then divides (2 to 5). At the same time the cytoplasm becomes constricted and finally two uninucleated daughter schizonts are formed (6, 7). This division is repeated. Some of the schizonts which contain two daughter nuclei (5) remain without cytoplasmic division and grow in size. In the mean time, each of the two nuclei undergoes division simultaneously so that a large elongated



Textfig. H. The life cycle of *Thelohania legeri*. Original body with four nuclei is produced (8, 9). Division takes place and two large schizonts are formed each with two nuclei (10). These nuclei lose their vesicular nature, become compact, and divide, the daughter halves being very often connected with each other with a strand (12, 13). By division two binucleated forms are formed; the nuclei are cousin nuclei and not daughter nuclei (14). The zygote or sporont is formed by the fusion of the two nuclei (15, 16). The nucleus now divides three times in succession producing stages with two, four and eight nuclei (17 to 25). Now the sporont is transformed into a pansporoblast with eight sporoblasts, each of which develops into a spore (26). When the spore reaches the gut of a new host larva, the filament extrusion and emergence of the sporoplasm as an amoebula take place and the development is repeated. During the nuclear fusion a large number of chromatic granules appear in the cytoplasm which apparently control the growth of the pansporoblast and the formation of the spore membrane.



Textfig I. The life cycle of *Stempellia magna*. Original.

In *Thelohania opacita* (Kudo 1924c) the development is in general similar to that of *T. legeri* mentioned here, but the two nuclei which by fusion become the sporont nucleus seem to be daughter nuclei and the chromatin granules which appear during the early phases of the sporogony are not so numerous as in *T. legeri*. Debaisieux (1919) and Debaisieux and Gastaldi (1919) observed similar development in the species of Thelohania parasitic in Simulium larvae. In a mictosporous form, *Stempellia magna* (Kudo, 1924b) development took place in the following manner. When the spore reaches the mid-gut of a new host culicine larva, it extrudes its polar filament (Textfig. I, 1) which later becomes detached. Through the foramen thus made, the apparently uninucleated sporoplasm creeps out as an amoebula (2). This amoebula enters an adipose tissue cell of the host and becomes a schizont (3). It grows and undergoes binary fission (4 to 9) and increases in number. As in the case of *Thelohania legeri*, the nucleus of the schizont divides without accompanying cytoplasmic constrictions so that binucleated bodies are formed (10 to 15). A growth period follows this and then again the nuclei divide; this stage is initiated by division of the karyosome and forms elongated bodies with four to eight nuclei (16 to 21). They divide into binucleated bodies (22, 23). The nuclei after casting off chromatin granules fuse into one and the sporont is formed (24 to 27). This sporont may directly transform itself into a single spore (28, 29, 41) or its contents may divide into two (30, 31 to 33), four (30, 34, 35 to 37) or eight sporoblasts (30, 34, 35, 38 to 40). Each sporoblast is uninucleated (41), but the nucleus seems to throw off a small chromatin granule which become lodged at one end of the body. The other nuclei breaks up into very minute chromatin granules which develop into a coiled polar filament (42) and the remaining chromatin granules reconstruct a nucleus.

RELATIONS BETWEEN MICROSPORIDIA AND THEIR HOSTS

MODES OF INFECTION

No intermediate host animals have up to date been found for Microsporidia. The infection of a new host animal takes place when the latter ingests spores of a specific microsporidian capable of germinating in its gut. In certain cases the spores seem to repeat the development in the same host in which they were formed. This auto-infection was considered by Zander (1911) and Fantham and Porter (1912a) for *Nosema apis*, Kudo (1916) for *N. bombycis* and Paillot (1918a) for *Perezia legeri*. Similar cases are also known to occur in Myxosporidia (Kudo, 1920a).

Infection through the reproductive organ of the hosts:—Since Pasteur (1870) established germinative infection in *Nosema bombycis*, it is an absolutely unquestioned fact that this is the case. Nöller (1912) noted that *Nosema pulicis* invaded not only the host digestive tract, but also ovary and ovarian eggs of *Ctenocephalus canis*, and suggested that the parasite was carried from generation to generation through germinative infection. Flu (1911) in *Musca domestica* and Chatton and Krempf (1911) in *Drosophila confusa* and *D. plurilineata* noted that *Octosporea muscae-domesticae* invaded the yolk of the egg, and suggested that there might be germinative infection. Pérez (1906) reported heavy infection of the ova of host crab (one specimen) by *Thelohania maenadis*, which showed absolute normality and "les ovules étaient chargés de vitellus, et selon toute vraisemblance auraient été prochainement pondus." In *Plistophora stegomyiae*, Marchoux and Salimbeni (1906) noticed that this mode of infection was quite frequently seen in host mosquitoes. According to Christophers (1901) and Nicholson (1921) the eggs of *Anopheles maculipennis* are frequently found loaded with a sporozoan. There seems to be little doubt in such a case the germinative infection will take place if the infection is moderate so that it does not affect the development of the host embryo.

Infection through the mouth: When taken into the alimentary canal of a specific host with the food, the spores germinate and initiate a new infection. This seems to be the ordinary method of infection in numerous species. It has been proved through experimental infection that the following Microsporidia invade the respective host *per os*: *Nosema bombycis*, *N. apis*, *N. bombi*, *N. ctenocephali*, *Thelohania legeri*, *T. opacita*, *Stempellia magna* and *Glugea anomala*.

EFFECT OF MICROSPORIDIAN INFECTION UPON THE HOST

When a host animal is more or less heavily infected by a microsporidian, it frequently shows external as well as internal changes due to the infection. Externally, the host may show changes in coloration, size, form or activity, all of which could clearly be recognized either by unaided eye or by a low magnification.

In numerous instances, especially when the hosts are small transparent aquatic animals, there have been noted striking changes in the coloration of the body due to localized or general microsporidian infections. In such cases the transparency of the body is lost and replaced by opacity. This state has been recognized in the following cases:

Hosts	*Microsporidia*
Gymnophallus somateriae strigatus	*Nosema legeri*
Glossiphonia complanata	*N. glossiphoniae*
Baetis nymph	*N. baetis*
Cyclops albidus	*N. infirmum*
C. viridis	*N. cyclopis*
Daphnia maxima	*Gurleya tetraspora*
Caddis-fly larvae	*G. legeri*
Diaptomus castor	*G. richardi* (Fig. 753)

Hosts	*Microsporidia*
Palaemon rectirostris and *P. serratus*	*Thelohania octospora*
Astacus fluviatilis	*T. contejeani*
Crangon vulgaris	*T. giardi*
Gammarus pulex	*T. mülleri* (Fig. 751)
Corethra plumicornis	*T. corethrae* (Figs. 755, 756)
Simulium reptans	*T. varians*
S. larvae	*T. bracteata*
	T. fibrata (Fig. 766)
	T. multispora (Fig. 765)
Anopheles larvae	*T. legeri* (Fig. 764)
	T. obesa
	T. pyriformis
Culex larvae	*T. opacita* (Fig. 763)
	Stempellia magna

In other cases, the infected part of the host body may show coloration different from the normal. In the silkworms infected more or less heavily by *Nosema bombycis*, a large number of dark brown spots appear (Fig. 757). The larvae of *Ctenocephalus felis* infected by *N. ctenocephali* showed a dark and mottled appearance by which Korke distinguished them easily from healthy larvae. In simulium larvae infected by *Plistophora simulii* form e, *e* Debaisieux and Gastaldi noted red coloration of the parasitic masses. The muscle of *Gammarus pulex* invaded by *Thelohania giraudi* was greyish yellow in color (Fig. 752). Frequently the host may show irregular dimensions as a result of infection. In the silkworms which are heavily infected by *Nosema bombycis*, the host larvae remain small so that the condition of infection is easily noted by a casual observer. In the case of *Culex territans* heavily infected by *Stempellia magna*, the host body is somewhat smaller than the normal ones. On the other hand Simulium larvae infected by a microsporidian are somewhat distended. The caddis-fly larvae infected by *Gurleya legeri* were swollen and showed congested appearance.

The activity of the host usually decreases as a result of heavy infection. Even when the muscular tissue is not directly infected as in the cases of *Nosema baetis*, *Gurleya legeri*, etc., the muscles are pushed aside on account of the great distension of the infected fat body, and this affects the movements of the host body. When the muscular tissue is the seat of infection, the host becomes inactive. This condition was seen in the following cases:

Hosts	*Microsporidia*
Bombyx mori	*Nosema bombycis*
Gymnophallus somateriae strigatus	*N. legeri*
Ctenocephalus felis	*N. ctenocephali*
Cyclops albidus	*N. infirmum*
Baetis sp.	*N. baetis*

Hosts	*Microsporidia*
Palaemon rectirostris	*Thelohania octospora*
P. serratus	*T. octospora*
Astacus fluviatilis	*T. contejeani*
Anopheles larvae	*T. legeri*
	T. obesa
Culex larvae	*T. opacita*
	Stempellia magna
Ephemera vulgata	*Telomyxa glugeiformis*

When the microsporidian infection is very intensive, the host is deformed. This deformity has long been known in silkworms infected by *Nosema bombycis* and in fish invaded by *Glugea anomala*. A glance at Fig. 761 explains how this happens in the latter case. I have repeatedly noticed conspicuous deformities in the mosquito larvae due to heavy infection by a microsporidian.

Delphy mentions a remarkable deformity of the posterior part of a fish infected by *Plistophora destruens*. Le Danois figures a *Crenilabrus melops* with a remarkable change in form due to an infection by *P. labrorum*. Strickland states that Simulium larvae heavily infected by *Thelohania bracteata* showed a distension of the abdomen. Cépède noted a *Cobitis barbatula* with a yellowish white ellipsoidal, but transparent tumor distending the integument below the lateral line near the vent and found that this was due to a heavy infection by *Plistophora macrospora*. Weissenberg found that the changes observed in a host fish of *Glugea hertwigi* were analogous to those of the host for *G. anomala*. As a rule, larger hosts do not show noticeable external symptoms of infection, although particular tissues may be heavily infected. The gills of a fish parasitised by *Nosema branchiale* showed numerous white spots (Fig. 762). The testis of *Barbus barbus* infected by *Plistophora longifilis* showed many whitish rounded spots of variable size on its surface (Fig. 760). The central nervous system of *Lophius piscatoris* when heavily parasitised by *Nosema lophii* shows conspicuous tumors (Fig. 758). The intestine of a fish host of *Glugea stephani* exhibited rounded bodies protruding from the gut wall into the coelom (Fig. 759). Smelts infected by *G. hertwigi* presented a typical appearance of the viscera (Fig. 767). The infection by *Glugea danilewskyi* of the amphibians or reptiles was characterized by whitish spots in the muscular tissue.

The members of the genus Glugea seem to attack certain host cells which develop into cysts of remarkable dimensions, the so-called "glugea-cysts". Leucocytes were reported as acting against the parasites in some cases. Sasaki (1897) observed active phagocytosis of spores of *Nosema bombycis* by its host cells. Mrázek and Caullery and Mesnil recognized similar action on the part of the hosts for *Nosema lophii* and *Glugea laverani* respectively. Weissenberg noticed that migratory cells of the host were seen in the cysts of *G. hertwigi* and spores were undoubtedly taken in by the former. I have made similar observations in the case of *Nosema baetis* (Kudo, 1921a). Léger observed that the fat body of infected hosts of *Thelohania varians* was smaller in size than in normal ones. Fantham and Porter recognized in the infection of *Nosema bombi* that besides the diminution of the fat body, an increase in hemocoelic fluid had occurred. In other cases where this tissue is the only seat of the microsporidian invasion, the fat body is completely replaced by the parasite and this causes the opacity of the infected area seen with unaided eyes. As a direct consequence, host animals in many cases succumb to death.

Henneguy and Thélohan stated that when attacked by *Thelohania octospora*, the muscle fibrillae of the host do not usually show any alteration. While elasticity is sometimes overcome and rupture results, the muscle fibrillae, however, remain exceedingly clear, no degeneration taking place. The nuclei of the infected muscle fibers are more numerous and smaller in size than in normal fibers. The muscular activity is considerably diminished. The infected animals do not survive long, all succumbing to death by the end of autumn. The same authors mentioned that the infected host of *T. contejeani* showed considerable diminution of muscular activity. In *Plistophora destruens*, Delphy noticed the degeneration of infected muscular tissue of the host fish. In the muscles of *Carcinus maenas* infected by *Nosema pulvis* and *Thelohania maenadis*, the muscle fibrillae become atrophied, yet some were perfectly normal and intact. The spores seemed to occupy the sarcoplasm and the latter to reabsorb the fibrillae.

To interprete the fact that no egg-bearing females were found among infected host animals, Henneguy and Thélohan expressed the opinion that *Thelohania giardi* and *T. octospora* probably cause parasitic castration in the host crustaceans, although the reproductive organs are not the seat of the infection. Pérez noticed that the female crab infected either by *T. maenadis* or by *Nosema pulvis*, suffered this effect more than the male, and in some cases the follicle cells reabsorbed the ova by phagocytosis. Léger and Duboscq stated that *Nosema frenzelinae* prevented the host gregarines from carrying on sexual development and called the phenomenon parasitic castration. Strickland did not see any reproductive organs in the host infected by *Thelohania bracteata*, *T. fibrata* or *T. multispora*. Simulium larvae infected by these parasites never pass through the pupal stage to the adult. According to Pérez, the blood of crabs infected by *Nosema pulvis* or *Thelohania maenadis* was milky in appearance and less coagulable during the schizogony of the parasite, due probably to the degeneration of numerous globules which appear, while the blood assumed normal appearance later.

One of the most conspicuous changes an infected host cell undergoes is the striking hypertrophy of its nucleus which may become enormously enlarged, may increase in number or may show various ways of division. The cytoplasm, of course, becomes enlarged because of the large number of parasites lodged inside. According to Schuberg, the most typical case of nuclear hypertrophy of an infected host cell was observed in *Plistophora longifilis* (Fig. 768). In the case of *Gurleya francottei*, the host epithelial cell becomes twice as broad as normal and the cytoplasm disappears. The host nucleus undergoes hypertrophy as the cell body becomes larger and shows ultimately chromatolysis and karyolysis simultaneously, i. e., the chromatic substance assembles in masses at the periphery of the nucleus, losing its characteristics. The cell membrane persists in spite of the enormous multiplication of the parasite in the cytoplasm (Fig. 776).

Léger and Hesse observed hypertrophy of the epithelial cells of the intestine of the host oligochaete infected by *Cocconema slavinae*. The same authors state that in *Mrazekia stricta* and *M. caudata* infected lymphocytes of the oligochaetes undergo marked hypertrophy, the diameter often exceeding 100μ, and the nuclei divide repeatedly. In *Thelohania chaetogastris*, Schröder notes that in the case of heavy infection, the nucleus of the host cell becomes surrounded by parasites and is taken into the cysts. Such a nucleus is vesicular, its network becomes coarse, and the chromatin granules are to be found mainly under the nuclear membrane. The karyosomes become larger and in many cases seem to divide repeatedly. The nucleus seems to break into two amitotically, and frequently forms rounded projections (Figs. 777-779). The same author further stated that *Nosema bryozoides* was responsible for the hypertrophy and amitotic division of the nucleus of the affected host cell. Mercier observed abnormal, asymmetrical, multipolar or confused mitosis of the adipose cell nucleus of the host caused by an infection of *Plistophora* sp. Debaisieux (1919) observed enlargement of the infected host cells and their nuclei which divided mitotically (Fig. 771). In simulium larvae infected by *Thelohania fibrata*, Debaisieux and Gastaldi stated that the nuclei of the host cells enclosed in the tumor were relatively numerous and voluminous and contain chromosomes which appeared as "piled dishes". The nuclei of the salivary gland cells, thus hypertrophied reached 170μ in diameter. The same authors further state that the nucleus of the adipose tissue cell and muscle cell infected by *T. bracteata*, become hypertrophied and altered. In the case of *Thelohania corethrae* parasitic in the oenocytes of the larva of *Corethra plumicornis* Schuberg and Rodriguez noticed distinct hypertrophy of the cell-body and nucleus of the infected cells.

I have observed enormous enlargement of the cell body and nucleus of the infected fat body of several mosquito larvae and others due to microsporidian infections such as in *Culex pipiens* parasitised by *Stempellia magna*, in *Baetis* sp. infected by *Nosema baetis*, etc. (Figs. 769, 770, 772-774).

It is a well known fact that the infection of *Nosema bombycis* often causes the death of a large number of infected silkworms, in which case the disease assumes a serious epidemic form. The larvae of *Bombyx mori* become undernourished, atrophy and finally die when infected more or less heavily by the microsporidian. They cannot spin cocoons since the silk glands are usually most heavily invaded by the parasite and this hinders the formation of silk in the glands. Even when cocoons are spun, the silk threads are not uniform in thickness, and easily broken during the reeling processes, owing to the abnormal condition of the glands. They usually die in the cocoons. The infected moths are generally weak and often cannot copulate. The females lay eggs of smaller number and of irregular form, in piles, while the normal moths would lay them in a uniform layer. When the maternal infection is heavy, the embryo cannot complete its development and dies inside the egg-chorion. The effect seems to be either mechanical or physiological, the lack of normal nutrition on the part of the host being the main cause of its death.

The stomach of hive bees infected by *Nosema apis*, becomes distended and white in color owing to the number of parasites present in the lumen as well as in the gut-epithelium. This seems to disturb the function of the digestive canal to such an extent that the death of the host results. However, there seem to be contradictory observations on this condition.

According to Henneguy and Thélohan the infection of *Thelohania contejeani* took an epidemic form among its hosts, *Astacus fluviatilis*, in certain districts of France and Strickland noticed that simulium larvae infected by *Thelohania bracteata* died more quickly than the normal ones in captivity; death is often due to skin rupturing. Pérez states that crabs parasitised by either *Nosema pulvis* or *Thelohania maenadis* died through hemorrhage during a period of several weeks to months. I observed that *Nosema infirmum* was apparently responsible for the death of its host, *Cyclops albidus*. In my opinion, the fatal results of heavy microsporidian infections in anopheline and culicine larvae are beyond doubt. Dollfuss writes that the heavy infection by *Nosema legeri* of a trematode of Donax always resulted in the death of the host, while Léger and Hesse mention that *Perezia lankesteriae* probably causes the death of its host gregarines.

SPECIFIC RELATION BETWEEN HOST AND MICROSPORIDIAN

There seems to be a definite relation between a microsporidian parasite and its host; and in numerous cases the microsporidian invades only a specific tissue cell of the host animal. In several cases, a microsporidian has been found to be parasitic in a parasitic protozoan or metazoan in all of which cases except one, it attacks the parasite and not the host of the latter. This interesting phenomenon was noted in the following cases:

Nosema marionis in *Ceratomyxa coris* (a myxosporidian) in *Coris julis* and *C. giofredi*
N. mystacis in *Ascaris mystax* in a domestic cat
N. distomi in *Distomum linguatula* (?) in *Bufo marinus*
N. balantidii in *Balantidium* sp. in *Bufo marinus*
N. frenzelinae in *Frenzelia conformis* (a gregarine) in *Pachygrapsus marmoratus*
N. legeri in *Gymnophallus somateriae strigatus* in *Donax vittatus*
Perezia lankesteriae in *Lankesteria ascidiae* (a gregarine) in *Ciona intestinalis.*

Léger and Duboscq held the two Microsporidia, parasitic in the gregarines as gregarinophilous which term seems to be well fitted; on the other hand in the case of *Gurleya francottei*, it affected only the gut epithelium of Ptychoptera larvae and did not attack the gregarine, *Pileocephalus striatus*, living in the same habitat. According to Guyénot and Naville, *Glugea danilewskyi* was found parasitic in both *Tropidonotus natrix* and its parasitic trematode; this makes the sole exception to the rule cited above.

I have called attention to the fact that the microsporidian parasites of mosquito larvae are specific to the genus of the host (Kudo, 1924, 1924a). *Thelohania legeri*, *T. obesa* and *N. anophelis* are, as far as the present studies indicate, exclusive parasites of mosquitoes belonging to the genus Anopheles, found in France and the United States; while *Thelohania opacita*, *T. rotunda*, *T. minuta* and *Stempellia magna*, are exclusive parasites of members of the genus Culex. The larvae of the two genera are found living together in small bodies of water, although their modes of existence differ to some extent. I have always found that the typical infection among them is true to the host genus. This generic specificity between the microsporidian parasites and their mosquito hosts appears to be analogous with the relation which exists between hemosporidian parasites of the higher vertebrates and adult mosquitoes of the genera Anopheles and Culex. The underlying cause of the specific relation between the Microsporidia and their hosts awaits future solution.

TOXIN

Whether the microsporidian infection is accompanied by the production of a toxin or not is not known. Mesnil, Chatton and Pérard (1913) write that one of the authors prepared at Banyuls-sur-mer glycerine extracts of the liver of *Cepola rubescens* which was heavily infected by *Glugea ovoidea* and injected the extracts in a rabbit, a mouse and a frog, all of which survived. Imms (1914) suggested in his paper on *Nosema apis* that "possibly also toxic substances are produced which hastened the bee's end."

IMMUNITY

Finally as to the immunity of the host, a few observers have brought up the question. Pasteur (1870), Bolle (1898), Stempell (1909) and others, remarked that the silkworms of Nipponese breeds seemed to be more resistant than those of European breeds to the invasion of *Nosema bombycis.* The practical silkworm breeders of Nippon hold a similar view. The results I obtained tend to show that this is true. As to the Nosema infection of hive bees, Fantham and Porter (1912) mention that certain breeds of the bees can survive when placed among infected breeds and Imms (1914) stated that evidences show that partial immunity of stocks occurs; such stocks might be difficult to diagnose, though they would at the same time act as sources of infection for susceptible colonies, while White (1919) denied this view by writing that several breeds did not show any difference in being attacked by the microsporidian. This question remains to be solved in the future.

DISTRIBUTION OF MICROSPORIDIA

ZOOLOGICAL DISTRIBUTION

Up to the present 178 species of Microsporidia including the doubtful forms all of which are recorded in this paper, have been observed by numerous investigators in different parts of the world. The hosts of Microsporidia which number at least 222 are distributed among the following Phyla: Protozoa, Plathelminthes, Bryozoa, Rotifera, Nemathelminthes, Coelhelminthes, Arthropoda and Chordata, of which the Phylum Arthropoda is represented by 149 host species, two-thirds of the total number of the hosts. In the following pages two lists are presented to show the general distribution of the organisms among various groups of animals and further the species of Microsporidia, their respective hosts and the seat of infection.

The Hosts of Microsporidia

Protozoa			4 host species
Sporozoa		3	
Ciliata		1	
Plathelminthes			8
Trematoda		4	
Cestoda		4	
Bryozoa			2
Rotifera			2
Nemathelminthes			2
Coelhelminthes			10
Arthropoda			149
Crustacea		36	
Arachnoidea		1	
Chilopoda		1	
Hexapoda		111	
Apterygota	1		
Archiptera	9		
Orthoptera	3		
Coleoptera	8		

Hymenoptera	12		
Siphonaptera	1		
Trichoptera	1		
Diptera	47		
Lepidoptera	29		
Chordata			45
Pisces		39	
Amphibia		1	
Reptilia		5	
Total			222

Host species		Seat of infection	Microsporidian
Protozoa Sporozoa Gregarinidia....	*Frenzelina conformis* in alimentary canal of *Pachygrapsus marmoratus*	cytoplasm	*Nosema frenzelinae*
	Lankesteria ascidiae. in intestine of *Ciona intestinalis*	cytoplasm	*Perezia lankesteriae*
Myxosporidia...	*Ceratomyxa coris* in gall bladder of *Coris julis* and *C. giofredi*	cytoplasm	*Nosema marionis*
Ciliata...........	*Balantidium* sp. in cloaca of *Bufo marinus*		*N. balantidii*
Plathelminthes Trematoda.......	*Brachycoelium* sp. in *Donax trunculus, Tellina fabula, T. teunis, T. solidula*	parenchyma	*N. legeri*
	Distomum linguatula (?) in intestine of *Bufo marinus*	vitellaria	*N. distomi*
	Gymnophallus somateriae strigatus in *Donax vittatus*	tissues of metacercaria	*N. legeri*
	Spec. ? in stomach of *Tropidonotus natrix*		*Glugea danilewskyi*
Cestoda..........	*Taenia bacillaris*	parenchyma, gonads, ova	Gen. inc. *helminthophthorus*

Host species		Seat of infection	Microsporidian
	T. denticulata	gonads, ova	Gen. inc. *helminthophthorum*
	T. expansa	gonads, ova	Gen. inc. *helminthophthorum*
	Sp.?	Parenchyma	Gen. et spec. incert. (Guyénot, Naville et Ponse)
Bryozoa	*Plumatella* (*Alcyonella*) *fungosa*	testis, body cavity	*Nosema bryozoides*
	P. repeus	body cavity	*Nosema bryozoides*
Rotifera	*Actinurus neptunius*		Gen. et sp. inc. (Fritsch)
	Asplanchna sp.		Gen. inc. *asplanchnae* Gen. inc. *polygona*
Nemathelminthes	*Ascaris mystax* in intestine of cat	female genital organ	*Nosema mystacis*
		parenchyma, gonads, ova	Gen. inc. *helminthophthorum*
	Protospirura muris in intestine of *Mus musculus*	epithelium of intestine	*Thelohania reniformis*
Coelhelminthes	*Chaetogaster diaphanus*	connective tiss., muscle	*T. chaetogastris*
	Limnodrilus claparedianus	lymphocyte	*Nosema ciliata*
	L. hoffmeisteri	gut, coelom	*Mrazekia mrazeki*
	L. sp.	spermatocyte, lymphocyte	*M. caudata*
	Lumbriculus variegatus	lymphocyte	*M. stricta*
	Scolelepis fuliginosa	epidermis, its derivatives, body cavity	*Glugea laverani*
	Scoloplos mülleri	coelom, tissue	*Glugea laverani*
	Slavina appendiculata	gut epithelium	*Cocconema slavinae*
	Tubifex tubifex	lymphocyte	*Mrazekia caudata*
Hirudinea	*Glossiphonia complanata*	muscle	*Nosema glossiphoniae*

Host species		Seat of infection	Microsporidian
Arthropoda Crustacea	*Asellus aquaticus*	peri-gastric fat body	*Mrazekia argoisi*
	Astacus fluviatilis	muscle	*Thelohania contejeani*
	Atyephira sp.		*Plistophora miyairii*
			Cocconema miyairii
	Balanus amaryllis	coelom	*Cocconema stempelli*
	Carcinus maenas	muscle	*Nosema pulvis*
		muscle, ovum	*Thelohania maenadis*
	Ceriodaphnia quadrangula	abdomen	Gen. et sp. inc. (Fritsch)
	C. reticulata	coleom	*Plistophora obtusa*
	Chydorus sphaericus	coleom	*Plistophora obtusa*
	Crangon vulgaris	muscle	*Thelohania giardi*
	Cyclops albidus	muscle, gonads, fat body	*Nosema infirmum*
	C. fuscus	muscle, gonads, fat body	*N. cyclopis*
	C. sp		*N. parva*
		fat body	*Thelohania virgula*
			Gen. inc. *schmeilii*
	C. strenuus		Gen. inc. *rosea*
	C. viridis (*C. gigas*)	fat body	*Thelohania acuta*
	Daphnia kahlbergiensis	abdomen	Gen. et sp. inc. (Fritsch)
	D. longispina	coelom	*Plistophora obtusa*
	D. magna	gut-epithel.	*P. intestinalis*
	D. maxima	hypodermal tissue	*Gurleya tetraspora*
	D. obtusa		*Plistophora obtusa*
	D. pulex	fat body	*Thelohania acuta*
		fat body	Gen. inc. *coccoidea*
		coelom	*Plistophora obtusa*
		gut-epithel.	*P. intestinalis*
	Diaptomus castor		*Gurleya richardi*
	D. gracilis		Gen. inc. *colorata*
	D. salinus		Gen. inc. *schmeilii*
	(*D. richardii*)		
	D. vulgaris (*D. coeruleus*)		Gen. inc. *schmeilii*
	Gammarus locusta	muscle	*Glugea mülleri*
	G. pulex	muscle	*Thelohania mülleri*
		muscle	*T. giraudi*
	Holopedium gibberum		Gen. inc. *holopedii*
	Limnetis sp.	hypodermal cell	Gen. inc. *coccoidea*

Host species		Seat of infection	Microsporidian
	Moina rectirostris	coleom	*Plistophora oblusa*
	Palaemon rectirostris	muscle	*Thelohania octospora*
	P. serratus	muscle	*Thelohania octospora*
	Palaemonectes varians	muscle	*T. macrocystis*
	Paradoxostoma sp.		Gen. et. sp. inc. (Müller)
	Polyphemus oculus	coelom	*Plistophora oblusa*
	Simocephalus retulus	coelom	*Plistophora oblusa*
	Talitrus (?) sp.	muscle	*Thelohania* sp. Mercier
Arachnoidea......	*Aranea diadema*	muscle	Gen. et sp. inc. (Leydig)
Chilopoda........	*Geophilus* sp.	gut	Gen. incert *geophili.*
Hexapoda........			
Apterygota.....	*Podura aquatica*	reproductive organ	Gen. inc. *thysanurae*
Archiptera......	*Ameletus hideus* (nymph)	fat body	*Thelohania mutabilis*
	Baetis pygmata (nymph)	fat body	*T. baetica*
	B. rhodani (nymph)	fat body	*Plistophora vayssierei*
	B. sp. (nymph)	fat body	*Nosema baetis*
	Ephemera sp. (nymph)	Intestine	*Nosema ephemerae* α, β
	E. vulgata (nymph)	gut-epithel.	*N. schneideri*
		fat body	*Stempellia mutabilis*
		fat body	*Telomyxa glugeiformis*
	Ephemerella ignata (nymph)	fat-body, muscle, conn. tissue	*Gurleya legeri*
	Potamanthus (?) sp. (nymph)	gonads, fat body	Gen. et sp. inc. (Pfeiffer)
	Termes lucifugus	coelom	*Duboscqia legeri*
Orthoptera.....	*Blatta orientalis*	fat body	*Plistophora* sp. Mercier
	Gryllus campestris		Gen. et sp. inc. (Vlacovich)
	Platycleis grisea (*Decticus griseus*)		Gen. et spe. inc. (Balbiani)
Coleoptera......	*Melasoma* (*Chrysomela*) *populi*	Malpighian tubules (?)	Gen. et sp. inc. (Pfeiffer)
	Ocypus olons		Gen. et sp. inc. (Frey et Lebert)
	Omophlus brevicollis	Malpighian tubules	*Thelohania cepedei*
	Otiorhynchus fuscipes	fat body	*Nosema longifilum*

Host species		Seat of infection	Microsporidian
	Statira unicolor	Malpighian tubules	Gen. et sp. inc. (Frenzel)
	Spec. ? (larva)		*Plistophora* sp. (Pfeiffer)
	Tribolium confusum	fat body	Gen. et sp. inc. (White)
	T. ferrugieneum.	fat body	Gen. et sp. inc. (White)
Hymenoptera...	*Apis florea*	gut, Malp. tub. (?)	*Nosema apis* (experim.)
	A. mellifica	gut, Malp. tubules gut, Malp. tub. (?) muscle	*Nosema apis* *N. bombi* (experim.) Gen. et sp. inc. (Leydig)
	Bombus agrorum	gut, Malp. tubules	*Nosema bombi*
	B. hortorum	gut, Malp. tubules gut	*Nosema bombi* *N. apis* (experim.)
	B. lapidarius	gut gut, Malp. tubules	*N. apis* (experim.) *N. bombi*
	B. latreillelus	gut, Malp. tubules gut	*N. bombi* *N. apis* (experim.)
	B. sylvarum	gut, Malp. tubules	*N. bombi*
	B. terrestris	gut, Malp. tubules gut	*N. bombi* *N. apis* (experim.)
	B. venustus	gut	*N. apis* (experim.)
	Mason bees	gut	*N. apis* (experim.)
	Vespa germanica	gut	*N. apis* (experim.)
	V. media		Gen. et sp. inc. (Pfeiffer)
Siphonaptera....	*Ctenocephalus canis*	gut, fat body, salivary gland Malpighian tubules	*Nosema pulicis*
	(*C. felis*)	ovary, gut	*N. ctenocephali*
Trichoptera.....	Sp. ?	fat body	*Gurleya legeri*
Diptera........	*Aëdes calopus* (larva)		*Nosema stegomyiae*
	(larva, adult)		*Plistophora stegomyiae*
	A. cantans (larva)		*Nosema* sp. Nöller
	A. nemorosus (larva)		*N.* sp. Nöller *Thelohania* sp. Nöller
	A. sp. (larva)		*N.* sp. Martini

Host species		Seat of infection	Microsporidian
	Anopheles bifurcatus (larva)		*Thelohania legeri*
	A. crucians (larva)	fat body	*Thelohania legeri*
	A. maculipennis (larva)	fat body	*Thelohania legeri*
	(adult)	ova	Gen. et sp. inc. (Christophers)
	A. punctipennis (larva)	fat body	*Thelohania legeri*
	A. quadrimaculatus (larva)	gut-epithel.	*Nosema anophelis*
		fat body	*Thelohania legeri*
		fat body	*T. obesa*
	(adult)	gut-epithel.	
		fat body	*Nosema anophelis*
		fat body	*Thelohania legeri*
	A. sp. (larva)	gut-epithel.	*Nosema anophelis*
		fat body	*Thelohania pyriformis*
	(adult)	ova	Gen. et sp. inc. (Christophers)
		coelom, gut	Gen. et sp. inc. (Grassi)
		ova	Gen. et sp. inc. (Grassi)
	Calliphora erythrocephala	gut	*Nosema apis* (experim.)
	Ceratopogon sp. (larva)	fat body	*Spironema octospora*
		fat body	*Toxonema vibrio*
	Chironomus plumosus (larva)	fat body	*Mrazekia brevicauda*
	C. sp. (larva)		*Nosema chironomi*
	Corethra plumicornis (larva)	oenocyte	*Thelohania corethrae*
	C. sp. (larva)		*T. braziliensis*
	Culex fatigans (imago)	nerve chord	Gen. et sp. inc. (Ross)
	C. leprincei (larva)	fat body	*Thelohania rotunda*
		fat body	*T. minuta*
	(pupa)	fat body, nerve chord, muscle	*T. minuta*
	C. pipiens (larva)		*Nosema culicis*
		fat body	*Stempellia magna*
			Thelohania sp. Iturbe et Gon.

Host species	Seat of infection	Microsporidian
C. territans (larva)	fat body	*Thelohania opacita*
	fat body	*Stempellia magna*
C. testaceus (larva)	fat body	*Thelohania opacita*
C. sp. (larva)	fat body	*Thelohania opacita*
Culiseta annulata		*T.* sp. Bresslau
Drosophila confusa	epithel., muscle of mid-gut, ova	*Octosporea muscae-domesticae*
	midgut-epithel.	*O. monospora*
D. plurilineata	midgut epithel., and muscle, ova	*O. muscae-domesticae*
	midgut-epithel.	*O. monospora*
Homalomyia scalaris	gut	*Thelohania ovata*
	gut-epithel.	*Octosporea monospora*
Limnophilus rhombicus (larva)	fat body	*Thelohania janus*
Melaphagus ovinus	gut	*Nosema apis* (experim.)
Musca domestica	gut	*Octosporea muscae-domesticae*
Orthocladius sp. (larva)	fat body	*Mrazekia bacilliformis*
Pachyrhina pratensis	fat body, muscle, conn. tissue	Gen. incert. *strictum*
Ptychoptera chrysorrhoea	mid-gut	Gen. et sp. inc. (Frenzel)
P. contaminata	gut-epithel.	*Gurleya francottei*
Simulium bracteatum (larva)	fat body	*Thelohania bracteata* *T. fibrata* *T. multispora*
S. hirtipes (?) (larva)	fat body	*T. bracteata* *T. fibrata*
S. maculata (larva)	fat body	*T. bracteata* *T. fibrata* *T. multispora* *Plistophora simulii*, γ, ϵ, δ,
S. ochraceum (larva)	fat body	*Thelohania bracteata* *T. fibrata* *Plistophora simulii* α, β
S. ornatum (larva)	body cavity	*Thelohania varians*
S. reptans (larva)	fat body	*Thelohania varians*

Host species		Seat of infection	Microsporidian
	S. venustum (larva)	fat body	*T. bracteata* *T. fibrata* *Plistophora simulii* α, β
	S. vittatum (larva)	fat body	*T. multispora*
	Stegomyia fasciata (larva, adult)		*Nosema stegomyiae* *Plistophora stegomyiae*
	S. sp. (adult)	nerve chord	Gen. et spec. inc. (Ross)
	Tanypus setiger (larva)	fat body	*Cocconema micrococcus*
	T. varius (larva)	fat body	*Thelohania pinguis*
	T. sp. (larva)	fat body	*Cocconema polyspora*
	Tanytarsus sp. (larva)	gut-epithel. fat body	*C. octospora* *Mrazekia tetraspora*
	Tipula oleracea	gut	*Nosema apis* (experim.)
Lepidoptera....	*Abraxas grossulariata* (larva)	gut	*N. apis* (experim.)
	Arctia caja (larva)	gut	*N. bombycis* (experim.)
	Attacus cynthia (larva)		*N.* sp. Ishiwata
	A. pernyi		Gen. et sp. inc. (Balbiani)
	A. yamamai		Gen. et sp. inc. (Balbiani)
	Bombycidae (larva)		*Nosema sabaunae*
	Bombyx mori	all tissues	*N. bombycis*
	B. neustria		*N. bombycis* (?)
	Brassolis astyra		*N. astyrae*
	Caeculia spp.		*N. caeculiae*
	Callimorpha jacobae	gut	*N. apis* (experim.)
	Catopsilia eubule		*N. eubules*
	Danais erippus		*N. erippi*
	D gilippus		*N. erippi*
	Dione juno		*N. junonis* α, β
	D. vanillae		*N. vanillae* α, β, γ
	Ephialtes angulosa (adult)		*N. ephialtis*
	Halesidotis sp.		*N. halesidotidis*
	Heliotis armigera		*N. heliotidis*
	Hydria sp.		*N. hydriae* α, β, γ
	Lophocampa flavostica		*N. lophocampae*

Host species		Seat of infection	Microsporidian
	Lyda nemoralis (larva)	silkglands, fat body, etc.	Gen. et sp. inc. (Kulagin)
	Mechanites lysimnia		*N. lysimniae*
	Micrattacus nanus		*N. micrattaci*
	Papilis pompejus		*N. junonis* α, β (exper.)
	Pieris brassicae (larva)	gut	*N. apis* (experim.)
		silkglands, Mal. tub.	*Perezia mesnili*
		fatbody, giant blood cell	*P. legeri*
	Porthesia chrysorrhoea	mid-gut	Gen. et sp. inc. (Frenzel)
	Scea auriflamma		*Nosema auriflammae*
	Zygaena filipendulae	fatbody, muscle, etc.	Gen. incert. *strictum*
Chordata			
Pisces…………	*Abramis brama* × *Leuciscus rutilus*	ovary	*Plistophora elegans*
	Acerina cernua	mesentery	*P. acerinae*
	Alburnus mirandella	ovary, ova	*P. mirandellae*
	Barbus barbus (*B. fluviatilis*)	testis	*P. longifilis*
		ovary	Gen. et sp. inc. (Keysselitz)
	Blennius pholis	muscle	*Plistophora typicalis*
	Callionymus lyra	muscle	*Glugea destruens*
	Cepola rubescens	liver	*G. ovoidea*
	Clupea pilchardus (*Alosa sardina*)	conn. tiss., heart muscle	*G. cordis*
	Cobitis barbatula	muscle	*Plistophora macrospora*
	Coregonus exiguus bondella	ova	*Thelohania ovicola*
	Coris julis (*Julis vulgaris*)	liver	*Glugea depressa*
	Cottus bubalis	muscle	*Plistophora typicalis*
	C. scorpius	muscle	*Plistophora typicalis*
	Crenilabrus melops	muscle	*P. labrorum*
	Dasyatis centrura	gut	Gen. et sp. inc. (Linton)
	Entelurus aequoreus	airbladder muscle	*Glugea acuta*
	Gadus aeglefinis	gills	*Nosema branchiale*

Host species		Seat of infection	Microsporidian
	G. luscus	muscle	*Glugea shiplei*
	G. pollachius	connect. tiss. of eyemuscle	*G. punctifera*
	G. sp.	muscle	*Plistophora* sp. Drew
	Gasterosteus aculeatus		*Glugea anomala*
	G. pungitus		*Glugea anomala*
		muscle	*Plistophora typicalis*
	Girardinus caudimaculatus	muscle, etc.	*Nosema girardini*
	Gobius minutus	subcut. conn. tissue	*Glugea anomala*
	Hippoglossoides limandoides	fin-muscle	*Plistophora hippoglossoideos*
	Leuciscus phoxinus (*Phoxinus laevis*)		Gen. et sp. inc. (Pfeiffer)
	Lophius budegassa spinola	nervous system	*Nosema lophii*
	L. piscatoris	nervous system	*Nosema lophii*
	Motella tricirrata	liver	*Glugea ovoidea*
	Mugil auratus	muscle	*Plistophora destruens*
	Nerophis aequoreus	conn. tissue of airbladder muscle	*Glugea acuta*
	Osmerus eperlanus		*Glugea hertwigi*
	O. mordax		*Glugea hertwigi*
	Pleuronectes flesus	gut-wall	*G. stephani*
	P. platessa	gut-wall	*G. stephani*
	Pseudopleuronectes americanus	gut-wall	*G. stephani*
	Rhombus triacanthus	liver	Gen. et. sp. inc. (Linton)
	Sciaena australis	ovary	*Plistophora sciaenae*
	Syngnathus acus	airbladder conn. tissue	*Glugea acuta*
Amphibia........	*Rana temporaria*	muscle	*Glugea danilewskyi*
		conn. tissue cell	Gen. et sp. incert. (Guyénot et Naville)
Reptilia........	*Chalcides tridactylus*	muscle (?)	*Glugea danilewskyi*
	Emys orbicularis	muscle	*Glugea danilewskyi*
	Lacerta sp.	muscle (?)	*Glugea danilewskyi*
	Tropidonotus natrix	muscle, conn. tissue	*Glugea danilewskyi*
	Zamenis gemonensis		Gen. inc. *heteroica*

As is indicated above, the majority of Microsporidia are found in specific situations of a single specific host species. Some interesting conditions are, however, found in certain instances.

1) *Nosema bombycis* attacks every tissue of all developmental stages of its host, *Bombyx mori.*

2) A microsporidian may be found parasitic in different hosts belonging to a genus. *Thelohania legeri* has been noted in five species of Anopheles mosquitoes, while *T. opacita* in three species of Culex mosquitoes. The host species of the two genera have been seen in many instances living side by side in the same waters, yet there has not been a single case of mixed infection discovered.

3) If a host is a parasite of other animals, the latter usually escape the invasion of the microsporidian. Such is the case with *Nosema frenzelinae, Perezia lankesteriae* or *Thelohania reniformis.* An exception to this is *Glugea danilewskyi* which attacks both the vertebrate host and a trematode living in one of them.

4) Under experimental conditions a microsporidian may infect animals quite different from its natural hosts as in the case of *Nosema apis.*

As to the distribution of a microsporidian in the host body the following peculiarities are noticed:

In a protozoan, the microsporidian lives in its cytoplasm and does not attack the host nucleus.

In Plathelminthes they are found in the parenchyma and further in the reproductive organs. The two species of Bryozoa were found to have the testes invaded by a microsporidian.

The lymphocytes, epithelium of gut and muscles are chief seats of infection in the annelids.

In Crustacea, muscles and fat body are most frequently infected, while the gut-epithelium was only once found to harbor a microsporidian. In Hexapoda, the fat body is the commonest seat of infection, although the gut-epithelium is also frequently infected.

Among vertebrate hosts, in fishes muscles and ovaries are most frequently noticed to be infected by Microsporidia of which the majority belong to either Glugea or Plistophora. In Amphibia and Reptilia, the muscles are almost exclusively the seat of the invasion of *Glugea danilewskyi.*

The fact that the adipose tissue of an arthropod harbors frequently a microsporidian, is due not only to its being a comparatively large tissue with an abundant supply of nutriment for the parasite, but also to the strikingly changed conditions resulting from a more or less heavy infection by a microsporidian; this easily attracts an observer's attention. As is discussed elsewhere, the microsporidian infection of a new host is in most cases established *per os,* and the spores germinate somewhere in the host digestive tract under the influence of the alimentary fluid, yet the infection of epithelial cells of the gut was observed only in a few cases and in others these host cells seem to possess power of resistance adequate to prevent the young amoebulae from entering them.

GEOGRAPHICAL DISTRIBUTION

The wide distribution of one and the same species of Microsporidia in different localities is seen in several species. *Nosema bombycis* has been known to occur in every country where its hosts, the silkworms, are reared. The same is true with *N. apis* of adult hive bees. In case the host is a fish, especially a marine fish, the migration of the host will probably be the reason for the occurrence of apparently one and the same species of the Microsporidia in different waters. The following are some of the typical examples.

Nosema lophii found in Austria, Italy, France, and England;

Glugea anomala found in France, England, Russia and Germany;

G. stephani found in France, England and the United States.

Some Microsporidia parasitic in other aquatic animals have been seen in different parts of the world. Prominent among them are

Nosema bryozoides from Russia, Turkestan and Germany;

Gurleya legeri from France and England;

Thelohania legeri from France and the United States.

On the other hand, some species are limited to a definite small area and appear only under certain conditions. Thus, according to Léger and Hesse, *Gammarus pulex* from still water was infected by *Thelohania giraudi*, while individuals from running water were attacked by *T. mülleri*. Schuberg and Rodriguez state that *T. corethrae* was found only in a limited pond during a brief period and Paillot found *Perezia mesnili* in the host larvae only in certain localities. There are apparently many factors which seem to influence the distribution of the organisms under consideration, of which little is known.

In the following are listed the names of countries where Microsporidia have been reported to occur.

Australia	*Nosema bombycis* *apis*	*Plistophora sciaenae*
Austria	*Nosema bombycis* *lophii*	*Nosema branchiale*
Belgium	*Glugea anomala* *danilewskyi* *mülleri* *Gurleya francottei* *Thelohania varians*	*Thelohania bracteata* *fibrata* *multispora* *Plistophora simulii* γ, δ, ε

Brazil	*Nosema bombycis* *vanillae* α, β, γ *astyrae* *girardini* *junonis* α, β *lysimniae* *eubules* *lophocampae* *erippi* *ephemerae* α, β *chironomi* *ephialtis* *balantidii* *stegomyiae*	*Nosema heliotidis* *halesidotidis* *caeculiae* *hydriae* α, β, γ *micrattaci* *sabaunae* *auriflammae* *mystacis* *distomi* *Thelohania braziliensis* *bracteata* *fibrata* *Plistophora stegomyiae* *simulii* α, β
Canada	*Nosema apis*	
Czechoslovakia	*Nosema ciliata*	*Mrazekia caudata*
Denmark	*Nosema apis*	
Dutch East Indies	*Octosporea muscae-domesticae*	
France	*Nosema bombycis* *parva* *marionis* *lophii* *pulvis* *longifilum* *frenzelinae* *schneideri* *legeri* *Glugea anomala* *destruens* *punctifera* *ovoidea* *acuta* *cordis* *depressa* *laverani* *stephani* *Perezia lankesteriae* *mesnili* *legeri* *Plistophora acerinae* *vayssierei* *macrospora* *intestinalis* *labrorum* sp. Mercier *destruens*	*Gurleya legeri* *richardi* *Thelohania giardi* *acuta* *virgula* *octospora* *contejeani* *mülleri* *varians* *pinguis* *janus* *maenadis* *legeri* *cepedei* sp. Mercier *giraudi* *Stempellia mutabilis* *Duboscqia legeri* *Plistophora typicalis* *obtusa* *mirandellae* *Mrazekia argoisi* *mrazeki* *caudata* *brevicauda* *stricta* *tetraspora* *bacilliformis*

	Cocconema micrococcus	*Octosporea muscae-domesticae*
	polyspora	*monospora*
	octospora	*Spironema octospora*
	slavinae	*Toxonema vibrio*
	stempelli	*Telomyxa glugeiformis*
Germany	*Nosema bombycis*	*Gurleya tetraspora*
	parva	*Thelohania acuta*
	bryozoides	*virgula*
	apis	*mülleri*
	pulicis	*chaetogastris*
	glossiphoniae	*corethrae*
	culicis	sp. Bresslau
	sp. Nöller	sp. Nöller
	Glugea anomala	*Plistophora obtusa*
	danilewskyi	*elegans*
	mülleri	*longifilis*
	hertwigi	
Great Britain	*Nosema lophii*	*Glugea shiplei*
	apis	*Gurleya legeri*
	bombi	*Thelohania octospora*
	Glugea anomala	*ovata*
	stephani	*Plistophora hippoglossoideos*
Iceland	*Plistophora* sp. Drew	
India	*Nosema bombycis*	*Nosema ctenocephali*
Italy	*Nosema bombycis*	*Glugea danilewskyi*
	lophii	*Thelohania macrocystis*
Natal	*Nosema apis*	
Nippon	*Nosema bombycis*	*Plistophora miyairii*
	sp. Ishiwata	*Cocconema miyairii*
Poland	*Glugea danilewskyi*	
Russia	*Nosema bryozoides*	*Glugea anomala*
Switzerland	*Nosema apis*	*Thelohania ovicola*
Turkestan	*Nosema bryozoides*	
The United States		
	Nosema apis	*Thelohania multispora*
	baetis	*opacita*
	cyclopis	*reniformis*
	infirmum	*mutabilis*
	anophelis	*baetica*

Glugea stephani
hertwigi
Thelohania legeri
bracteata
fibrata
Thelohania obesa
pyriformis
rotunda
minuta
Stempellia magna

SEASONAL DISTRIBUTION

At present one possesses very little data on which a discussion of this subject can definitely be based. Henneguy and Thélohan (1892a) stated that *Thelohania octospora* appeared in the host prawns from March to April, that it occurred abundantly in July and August decreasing toward September and October, and that the hosts disappeared entirely after November 15. Pérez (1905) saw that the incidence of infection of *Carcinus maenas*, a crustacean, by *Thelohania maenadis* and *N. pulvis* became higher from January to June. The results he recorded are as follows:

Date	Number of crabs examined	Number of host crabs infected by *T. maenadis*	Number of hosts infected by *N. pulvis*
January 24	263	1	–
March 19	53	1	1
26	21	1	–
June 5	136	15	–
9	134	16	3
21	113	26	5

Brug (1914) stated that all the larvae of *Homalomyia scalaris* studied in November were infected by *Octosporea monospora*, while in January and February the infection was less intensive. White (1919) carried a very careful study on the seasonal distribution of the honey bees in an apiary infected by *Nosema apis* from April, 1912, to June, 1915, and concluded that the number of infected bees found at different periods of the year varied considerably. April and May furnished the highest percentage, being 18 and 17 per cent respectively. In March, June, July, August and September the number of Nosema-infected bees among those examined was 11, 8, 7, 5 and 10 per cent respectively. In the case of *Nosema bombycis*, the per cent of infection in the host silkworms is highest in summer months, while the spring or fall breed is usually less heavily infected. The seasonal distribution is without doubt correlated with the host's metabolism which is accelerated among other things, by the rise in atmospheric temperature. It seems to be general in nature that the incidence of microsporidian infection is high in summer and low in winter.

TECHNIQUE

Study of fresh material.—This is by all means the most important method as fixation and staining may frequently cause morphological changes of the comparatively delicate vegetative forms. It is desirable to study the microsporidian in the host tissue, the tissue fluid, or in the body fluid in which the infected tissue or organ is suspended. Distilled water or physiological salt solution may well be used with great care as they often cause more or less serious changes in the parasite, such as in the form and structure of the vegetative form, the extrusion of the polar filament of the spore, etc.

When the fresh spores are available for study, it is easy to determine whether or not the spores belong to a microsporidian. Under a low magnification microsporidian spores appear uniform in size, shape and appearance, are highly refractive and are as a rule heavier than other matters present in the field at the same time so that they are always found in the lowermost focal plane. A clear space or vacuole is found in many species at one pole of the spore, but it cannot be depended upon as a characteristic of a microsporidian spore, since similar structure has been noticed in spores of haplesporidians or in yeast cells. For casual observers some of the plant cells may appear as microsporidian spores, therefore, in examination of host animals for the Microsporidia, one must use precaution not to confuse other animal as well as plant cells with them. Sturtevant (1919) reports that the pollen grains of corn contained oval starch granules similar in appearance and size to the spores of *Nosema apis*. In such case, of course, an addition of iodine solution to the preparation would prove the real nature of the objects. To distinguish a microsporidian spore from a yeast cell, Mercier recommends staining with Ziehl's fuchsin followed by decoloration with a weak sulphuric acid solution. Then yeast cells are decolorized, while microsporidian spores remain bright red. When fresh spores are available, further, the presence of a polar filament in the spore, which is the only proof of the spore belonging to the Microsporidia, is easily tested by using one of the methods mentioned elsewhere. The most convenient and satisfactory method of filament extrusion is by mechanical pressure. For this I recommended the following procedure (Kudo, 1921): "A very small drop of water emulsion of fresh microsporidian spores is placed upon a slide. It is desirable to have the outer margin of the cover glass unoccupied by the emulsion. Place the slide on a smooth and steady surface, and cover the cover glass with a piece of cloth or filter paper, over which the elbow is gently applied. Give a strong downward push to the arm. This will instantly cause the extrusion of the polar filament." The slide now can be examined under a dark field microscope. To make the preparation permanent the cover glass must be lifted up carefully. After being treated with fixatives, the smear is stained with Fontana's mixtures.

Study of fixed material.—The fixatives and staining methods ordinarily used for cytological study are employed here also. As fixatives, authors have used, 1) so-called Schaudinn's fluid (2 parts, cold saturated solution of corrosive sublimate + 1 part, absolute alcohol + a trace, glacial acetic acid), 2) Flemming's weak and strong fluids, 3) Sublimate acetic (saturated sublimate +5 per cent. acetic acid), 4) Alcohol acetic (absolute alcohol 95 cc. + glacial acetic acid 5cc), 5) Picro-formol, 6) Bouin's picro-formol-acetic acid, etc. For staining, 1) Heidenhain's iron haematoxylin, 2) Giemsa's fluid, 3) Delafield's haematoxylin, 4) Dobell's alcoholic iron haematein, 5) Picro-carmin, 6) methylene blue, 7) silver impregnation method, etc.

GLOSSARY

Amoebula stage. The sporoplasm which by amoeboid movements has left the spore membrane, a stage leading up to the schizont.

Anterior end. The end of the spore from which the polar filament becomes extruded through the foramen. If the two extremities are dissimilar in form, the anterior end is usually more or less attenuated.

Autogamy. Fusion of the two daughter nuclei to form a zygote or sporont.

Disporoblastic. Producing two sporoblasts as in Glugea, Perezia.

Disporous. Producing two spores as in Glugea, Perezia.

Karyogamy. The union of two gametes whose nuclei undergo intermingling.

Meront. Coined by Stempell (1902) to designate a schizont of a microsporidian.

Mictosporoblastic. Producing a variable number of sporoblasts as in Stempellia, Telomyxa.

Mictosporous. Producing a variable number of spores as in Stempellia, Telomyxa.

Monosporoblastic. Developing into a single sporoblast as in Nosema.

Monosporous. Developing into a single spore as in Nosema.

Octosporoblastic. Producing eight sporoblasts as in Thelohania.

Octosporous. Producing eight spores as in Thelohania.

Pansporoblast. Coined by Gurley (1893) to designate in a myxosporidian trophozoite an enclosed area in which two sporoblasts become differentiated. Strictly speaking, therefore, the genera Glugea and Perezia have pansporoblasts in this sense. The term however has also been used to designate in general a grown sporont of the polysporous genera in which two to many sporoblasts are formed.

Planont. The stage between free amoebula and schizont stages which are found in the alimentary canal or body cavity of the host soon after the spores germinate; coined by Stempell (1909).

Polar capsule. A sac in which the polar filament is coiled. One of the typical structures of a cnidosporidian spore.

Polar filament. A fine and long filament coiled in the polar capsule, which under suitable stimulation is extruded.

Polysporoblastic. Producing numerous sporoblasts as in Duboscqia, Plistophora.

Polysporous. Producing many spores as in Duboscqia, Plistophora.

Posterior end. The end of a spore opposite the anterior. If the two extremities are dissimilar in form, this is more or less rounded.

Schizogony. The changes which a schizont undergoes during its asexual reproduction.

Schizont. Early intracellular stages which multiply by asexual reproduction.

Spore-membrane. The envelope of a spore composed of a single piece or in some cases of two valves. Sporocyst.

Sporoblast. A cell which develops directly into a spore.

Sporogony. The changes in the development of spores from the sporont stage.

Sporont. An individual which gives rise to one to many sporoblasts.

Sporoplasm. The sporozoite of a cnidosporidian spore, a protoplasmic mass found inside of the spore.

Synkaryon. The sporont nucleus, which was formed by karyogamy.

Tetrasporoblastic. Producing four sporoblasts as in Gurleya.

Tetrasporous. Producing four spores as in Gurleya.

PART II TAXONOMY

CLASSIFICATION

Thélohan (1892) distinguished Microsporidia under the family name Glugeidae ("Glugéidées") from Myxosporidia by the following characters: Spores pyriform; a single polar capsule with pointed extremity; one clear vacuole, noncolorable with iodine, at the large extremity, and divided it into the following subdivisions.

Spores developed in each sporoblast.

- (1) Eight in number........................Genus Thelohania
- (2) variable in number: sporoblast
  - (a) isolated; each formed from a special small protoplasmic body; with persistent membrane..Parasite of muscles of Cottus.
  - (b) Formed in the interior of endoplasm of a plasmic body; with membrane disappearing after the formation of spores..............................Genus Glugea.

Gurley (1893) coined a new Order name Cryptocysts for Microsporidia and defined and subdivided it as follows:

Cryptocysts ord. nov. Myxosporidia in which the pansporoblast produces many (at the fewest 8) spores; the last minute, without distinct symmetry, with a single capsule; type (and only) family,......................Glugeidae fam. nov.

Glugeidae fam. nov.

Cryptocysts destitute of a bivalve shell; with the capsule at the anterior extremity and with an aniodinophile vacuole; type genus, ...Glugea Thélohan.

Glugea Thélohan 1891

Glugeidae possessing a myxosporidium, and in which the pansporoblast produces an inconstant but large number (always more than 8) of spores; pansporoblast membrane not subpersistent; type, *G. microspora* Thél. (Synonym for *G. anomala* Moniez).

Pleistophora gen. nov.

Glugeidae destitute of a myxosporidium and in which the pansporoblast produces an inconstant but large number (always more than 8) of spores; pansporoblast membrane subpersistent (as a polysporophorous vesicle); type (and only) species, ...*P. typicalis* sp. nov.

Thelohania Henneguy 1892

Glugeidae destitute of a myxosporidium and in which the pansporoblast produces constantly 8 spores; pansporoblast membrane subpersistent (as an octosporophorous vesicle); type, ...*T. giardi* Henneguy.

In his splendid work on Myxosporidia, Thélohan (1895) revised his Glugeidae as follows:

Glugeidae. Spores ordinarily small ovoid with a clear vacuole at the large extremity; at the opposite extremity a polar capsule with a filament, which is ordinarily totally invisible in fresh state. The spore membrane is bivalve, the sutural line is very difficult to observe. It is clearly noticeable in *Thelohania giardi*.

I Glugea. Myxosporidia generally parasitic in tissues (one exception in *G. marionis*). Spores are formed in the sporoblasts which become differentiated in the endoplasm; ovoidal, a clear vacuole at the large extremity; capsule invisible in fresh state; filament very long.

- a Spores ovoidal, short; breadth:length = 1:1.5; anterior extremity slightly attenuated: type, *G. microspora*.
- b Spores similar to a), but anterior extremity greatly attenuated: type, *G. acuta*.
- c Spores elongated ovoidal; breadth:length = 1:2.5: type, *G. marionis*

II Pleistophora. Myxosporidia in form of small spherical vesicles surrounded by a thin membrane of double contour and producing spores of variable and considerable number. *Pl. typicalis*.

III Thelohania. Myxosporidia closely related to the last: small vesicles, spherical or fusiform, containing eight spores. *T.octospora*.

It is strange to note that Thélohan although working in the laboratory of Balbiani who proposed the name of Microsporidia for these minute protozoons, did not use the term in his taxonomic considerations of the group. The name, Microsporidia, is indeed well fitted one for this group of Sporozoa in view of the recent findings by Léger and Hesse (1921, 1922) of various forms of minute dimensions. In this regard, Labbé (1899) was more farsighted, although he did not study Microsporidia himself, for he recognized the order Microsporidia as equivalent to the order Myxosporidia. Labbé's system is as follows:

Microsporidia. Spore with a single polar capsule which is invisible in fresh state without reagent. Spore very small.

Family Nosematidae. Spore generally very minute with a clear vacuole at one extremity and with a polar capsule invisible in fresh state at the other extremity. Spore membrane is probably bivalve.

Differentiation of the genera:

I	Sporoblast without envelope	1 Nosema
	Sporoblast with envelope	II
II	Spore in variable number	2 Plistophora
	Spore in constant number (8)	3 Thelohania

After studying some Cnidosporidia, Doflein (1899) offered the following classification.

Cryptocysts. Spores small and with hardly visible polar capsule. Four to many spores formed in a pansporoblast. Cell-parasites.

- (a) Oligosporogenea. 4 to 8 spores formed in each pansporoblast. (Thelohania, Gurleya).
- (b) Polysporogenea. Many spores in each pansporoblast. (Pleistophora, Glugea).

In 1905, Pérez revised Doflein's system and proposed the following:

Suborder Cryptocysts. Spore small, pyriform, with a polar capsule, visible only after treatment with reagents:

Section a. Polysporogenea. The trophozoite produces numerous spores by endogenous manner.

1 Vegetative nuclei bud in plasmodial layer which surrounds masses of spores ....Glugea

2 Vegetative nuclei mixed with spores in the endoplasm of trophozoite. Ectoplasm with immobile ciliary prolongations. ....Myxocystis

Section b. Oligosporogenea. The trophozoite transforms into a pansporoblast, containing a certain number of spores.

3 N spores.. Plistophora

4 8 spores.. Thelohania

5 4 spores .... Gurleya

Section c. Monosporogenea. The sporozoite transforms into a spore. Diffuse infiltration .... Nosema

The above quoted systems are based upon the modes of spore-formation. On the other hand, Stempell (1909) after making painstaking studies of *Thelohania mülleri* (1902), *Glugea anomala* (1904) and *Nosema bombycis* (1909) came to the conclusion that an adequate system could only be established when the form and development of the vegetative form, the mode of spore-formation and the form of spores are considered as the characters of family, genus and species respectively, and proposed the following classification:

Microsporidia

1 Nosematidae. Vegetative stage: intracellular uninucleate meronts capable of division.
  - Nosema. From each meront is formed a spore.
  - Thelohania. Each meront by division forms sporonts; each sporont gives rise to eight spores.
  - Gurleya. Each sporont forms four spores.

2 Plistophoridae. Fully developed vegetative stage is multinucleated meront, often capable of amoeboid movement.
  - Plistophora. The vegetative stage transforms into rounded sporont, from which many spores are formed.
  - Mariona n. gen. The spores are formed by endogenous budding in the cytoplasm of vegetative stage capable of amoeboid movements.
  - Myxocystis. The spores are formed by endogenous budding in the cytoplasm of vegetative stage, the ectoplasm of which possesses immobile cilia.

3 Glugeidae. Vegetative stage multinucleated, immobile, nondividing form which becomes encysted. The sporonts are formed by endogenous budding.
  - Glugea. Number of spores formed in each sporont variable.
  - Duboscqia. Each sporont gives rise to 16 spores.

Poche (1913) adopted this system by adding Telomyxidae (Léger et Hesse) to it. Recent investigations, however, brought out the fact that the so-called Glugea-cysts and the trophozoites of the genus Myxocystis are in reality hypertrophied host cells and are not any part of the Microsporidia. The genus Mariona now has become synonymous to the genus Nosema through Stempell's more recent study (1919). Therefore the system based upon the vegetative stages of Microsporidia possesses little significance at present.

In the allied Myxosporidia, the most satisfactory classification is based upon the form and structure of the spores (Kudo, 1920a). On the other hand, up to 1910, the microsporidian spores showed very little variations, being oval, pyriform or oblong, although spherical form was noted in a single species by Pérez in 1905. For this reason and also due to the obscurity of its structure and its minuteness, attempts to use the spore as the basis of classification have failed. Thélohan (1895), as is quoted above, subdivided the genus Glugea into three groups on the form of the spore. Lutz and Splendore (1903) attempted to differentiate the various forms of "Nosema" which they observed in Brazil by arranging them according to the forms of the spores, but later gave up the scheme (1904, 1908).

In 1910 Mrázek observed a microsporidian possessing cylindrical spores. Since that time similar spores were found by Flu (1911), Chatton and Krempf (1911) and Léger and Hesse (1916, 1922). The latter two authors found later spherical spores (1921), bacilliform and semicircular forms (1922). In bringing the genus Octosporea into Microsporidia, Chatton and Krempf (1911) stated that the number of spores formed in each pansporoblast cannot be used any more as a good basis of classification in Microsporidia, since there had been seen a number of species such as *Thelohania janus*, *T. cepedei*, *Stempellia mutabilis*, *Telomyxa glugeiformis*, *Octosporea muscae-domesticae* in which different sporeformations were noted.

Léger and his associates established previously the genera Perezia (Léger and Duboscq, 1909), Mrazekia (Léger and Hesse, 1916), Stempellia and Telomyxa (Léger and Hesse, 1910) on the number of spores formed in each sporont, but recently depended solely upon the form and the structure of the spore and disregarded entirely the sporogonic characters, in creating the genera Cocconema (1921), Toxonema and Spironema (1922) and proposed the following system which is based upon the form of spores and which therefore is fundamentally different from any previous ones (1922).

Dicnidea. Microsporidia the spores of which possess two polar capsules.
  Telomyxidae. Telomyxa.
Monocnidea. Microsporidia the spores of which possess one polar capsule.
  Glugeidae. Spores pyriform. Glugea, Nosema, Pleistophora, etc.
  Cocconemidae. Spores spherical. Cocconema.
  Mrazekidae. Spores straight, arched or spiral club-form. Mrazekia, Octosporea, Toxonema.

I believe the last mentioned system of Léger and Hesse is best fitted to present state of knowledge on Microsporidia, since 1) it is based upon the spore which is the most conspicuous part of a microsporidian life-cycle and in which a lesser degree of variation is noticed within one and the same species than in any other stages; 2) not only the form but also the structure of the spore can be used for differentiation of the species; 3) knowledge of the schizogony is still highly unstable as viewed in the light of the more recent investigations of Debaisieux, Weissenberg, etc.; and 4) as Chatton and Krempf remarked, there are numbers of forms in which mictosporous characters have been noticed. For these reasons, I have followed Léger and Hesse's new system with slight modifications.

Order MICROSPORIDIA Balbiani 1882

Intracellular parasites of typically invertebrates. Multiplication by schizogonic divisions. Sporont develops into one to numerous spores. The minute spore is covered with a resistant membrane, possesses a sporoplasm and one, or rarely two, comparatively long filaments which are coiled in a polar capsule that is usually obscure in the fresh state.

Suborder MONOCNIDEA Léger et Hesse 1922

Microsporidia, the spore of which is provided with one polar filament that is typically coiled in a polar capsule.

Family NOSEMATIDAE Labbé 1899

Spores oval, ovoid or pyriform. If subcylindrical, length is less than 4 times the breadth.

Genus NOSEMA Nägeli 1857 emend. Pérez 1905

1857	*Nosema*	Nägeli	1857 : 760
1895	*Glugea* (part.)	Thélohan	1895 : 356
1897	*Myxocystis*	Mrázek	1897 : 1-5
1899	*Nosema* (part.)	Labbé	1899 : 105-108
1905	*Nosema*	Pérez	1905 : 17
1910	*Myxocystis* (part.)	Mrázek	1910 : 245-259

Each sporont develops into a single spore. Type species: *Nosema bombycis* Nägeli.

The genus Myxocystis was established by Mrázek (1897) for a microsporidian which was found in the body cavity of *Limnodrilus claparedianus* and which showed what the author thought ciliated trophozoites. Mrázek created the genus because "it is difficult to place the species in any one of the known genera." Hesse (1905) distinguished the genus from the genus Glugea by "bodies covered by ciliary prolongations." Mrázek (1910), however, noticed that the so-called Myxocystis is not an independent organism, but simply a host cell, particularly the lymphocyte, infected by a microsporidian. The first form observed by Mrázek has oval spores, while the second one possesses tubular spores. Therefore, the genus Myxocystis is here considered as synonymous with the genera Nosema and Mrazekia.

Genus GLUGEA Thélohan 1891 emend. Weissenberg 1913

1891	*Glugea*	Thélohan	1891 : 29
1892	*Glugea*	Thélohan	1892 : 174
1893	*Glugea*	Gurley	1893 : 409
1895	*Glugea*	Thélohan	1895 : 355
1899	*Nosema* (part.)	Labbé	1899 : 105-108
1905	*Glugea*	Pérez	1905 : 17
1913	*Glugea*	Weissenberg	1913 : 126-131, 152
1920	*Glugea*	Debaisieux	1920 : 237
1921	*Glugea*	Weissenberg	1921 : 420

Each sporont develops into two spores. Host cells become enormously hypertrophied, forming the so-called Glugea-cysts. Type species: *Glugea anomala* (Moniez) Gurley.

The genus was established by Thélohan (1891) for the microsporidian parasite of Gasterosteus. Because of the conspicuous "cysts" in which large number of spores are contained, the polysporous definition of the genus was followed by many taxonomists. Weissenberg (1913) made a careful study of *Glugea anomala* and *G. hertwigi* and noticed that spore-formation takes place in the peripheral layer of the cysts and that each vacuole-cell divides into two sporoblasts. Debaisieux (1920) who studied in details the development of *G. anomala*, *G. danilewskyi*, *G. mülleri*, stated that "the genus Glugea is characterized by the formation of two spores from a zygote (sporont)." Weissenberg (1921) later definitely proved through experimental infection of young host fish of *G. anomala* that the so-called vegetative nuclei were none other than the host cell nuclei. Thus our present conception of the developmental stages of the genus is fundamentally different from the old notion. In view of the studies of Weissenberg and Debaisieux on the type species of the genus, I consider that the diagnosis of the genus should be changed from polysporous to disporous. And as such the genus is moved into a place next to the genus Nosema.

Genus PEREZIA Léger et Duboscq 1909

1909	*Perezia*	Léger and Duboscq	1909b: LXXXIX-XCV

Each sporont forms two spores. Host cell is not hypertrophied as in the last mentioned genus. Type species: *Perezia lankesteriae* Léger et Duboscq.

Genus GURLEYA Doflein 1898

1898	*Gurleya*	Doflein	1898 : 290-291

Each sporont produces four sporoblasts and ultimately develops into four spores. Type species: *Gurleya tetraspora* Doflein.

Genus THELOHANIA Henneguy 1892

1892	*Thelohania*	Henneguy in Thélohan	1892 : 174
1893	*Thelohania*	Gurley	1893 : 410
1895	*Thelohania*	Thélohan	1895 : 361
1899	*Thelohania*	Labbé	1899 : 111, 112
1905	*Thelohania*	Pérez	1905 : 17

Each sporont develops into eight sporoblasts and ultimately into eight spores. The sporont membrane may degenerate at different times of development. Type species (proposed by Gurley): *Thelohania giardi* Henneguy.

Genus STEMPELLIA Léger et Hesse 1910

1910	*Stempellia*	Léger and Hesse	1910 : 412

Each sporont develops into one, two, four or eight sporoblasts and ultimately into one, two, four or eight spores. Type species: *Stempellia mutabilis* Léger et Hesse.

Genus DUBOSCQIA Pérez 1908 emend.

1908	*Duboscqia*	Pérez	1908 : 631-633
1909	*Duboscqia*	Pérez	1909 : 18

Each sporont develops into 16 sporoblasts and ultimately into 16 spores. Type (and only) species: *Duboscqia legeri* Pérez.

It seems to be most probable that the "budding nuclei" of the so-called trophozoites described by Pérez are hypertrophied host cell nuclei, in the light of the findings of Mrázek (1910), Weissenberg (1913, 1921) and Debaisieux (1919, 1920).

Genus PLISTOPHORA Gurley 1893

1893	*Pleistophora*	Gurley	1893 : 410
1895	*Pleistophora*	Thélohan	1895 : 360
1899	*Plistophora*	Labbé	1899 :108
1905	*Plistophora*	Pérez	1905 : 17
1910	*Plistophora*	Schuberg	1910 : 422

Each sporont develops into many (more than 16) spores. Type species: *Plistophora typicalis* Gurley.

Gurley translated *ει* into *ei*, hence Pleistophora, which should be *i* according to the International Rules of Zoological Nomenclature. Labbé first corrected it.

Family COCCONEMIDAE Léger et Hesse 1922

Spores spherical or subspherical.

Genus COCCONEMA Léger et Hesse 1921

1921	*Cocconema*	Léger and Hesse	1921 : 1419-1421
1922	*Cocconema*	Léger and Hesse	1922 : 329

Spore spherical or subspherical. Type species: *Cocconema micrococcus* Léger et Hesse.

Family MRAZEKIDAE Léger et Hesse 1922

Spores tubular or highly cylindrical (length is greater than 5 times the breadth).

Genus MRAZEKIA Léger et Hesse 1916

1916	*Mrazekia*	Léger and Hesse	1916 : 346
1922	*Mrazekia*	Léger and Hesse	1922 : 327

Spores straight tubular. The filament possesses a rod-like basal portion. Type species: *Mrazekia argoisi* Léger et Hesse.

Genus OCTOSPOREA Flu 1911 emend. Chatton et Krempf 1911

1911	*Octosporea*	Flu	1911 : 530-533
1911	*Octosporea*	Chatton and Krempf	1911 : 178

Spores cylindrical; more or less arched; ends similar. Type species: *Octosporea muscae-domesticae* Flu.

Genus SPIRONEMA Léger et Hesse 1922

1922	*Spironema*	Léger and Hesse	1922 : 328

"Spores tubuleuses, tordues en hélice et aplaties du côté de l'axe d'enrolement." Capsule occupies the larger part of the spore. Without visible basal portion in the filament. Type (and only) species: *Spironema octospora* Léger et Hesse.

Genus TOXONEMA Léger et Hesse 1922

1922	*Toxonema*	Léger and Hesse	1922 : 328

Spores very small and arched or curved in semicircle. Type (and only) species: *Toxonema vibrio* Léger et Hesse.

Suborder DICNIDEA Léger et Hesse 1922

Spore with two polar capsules, one at each end, containing a polar filament.

Family TELOMYXIDAE Léger et Hesse 1910

With the characters of the suborder.

Genus TELOMYXA Léger et Hesse 1910

1910	*Telomyxa*	Léger and Hesse	1910 : 413-414
1922	*Telomyxa*	Léger and Hesse	1922 : 329

Spore with two polar capsules. Each sporont develops into 8, 16 or n spores. Type (and only) species: *Telomyxa glugeiformis* Léger et Hesse.

DESCRIPTION OF THE SPECIES

Order MICROSPORIDIA Balbiani 1882

The diagnosis of the order is given on page 65.

Suborder MONOCNIDEA Léger et Hesse 1922

The characters of the suborder are given on page 65.

Family NOSEMATIDAE Labbé 1899

The characters of the family are described on page 65.

Genus NOSEMA Nägeli emend. Pérez

The characters of the genus are described on page 65.
Type species: *N. bombycis* Nägeli 1857.

NOSEMA BOMBYCIS Nägeli 1957

[Figs. 1-39, 757; textfigs. B2, D]

1857	*Nosema bombycis*	Nägeli	1857 : 760
1858	*Panhistophyton ovatum*	Lebert	1858 : 149-186
1860	Pebrine corpuscles	A de Quatrefages	1860 : 1-638
1870	Pebrine corpuscles	Pasteur	1870 : 1-327
1884	Microsporidie	Balbiani	1884 : 150-168
1887	Pebrine corpuscles	Wood Mason	1887 : 1-3
1888	Pebrine corpuscles	Pfeiffer	1888 : 469-486
1894	*Glugea bombycis*	Thélohan	1894 : 1425-1427
1895	*Glugea bombycis*	Thélohan	1895 : 357-358
1900	*Glugea bombycis*	Toyama	1900 : 1-40
1907	*Nosema bombycis*	Léger and Hesse	1907 : 6
1909	*Nosema bombycis*	Stempell	1909 : 281-358
1910	*Glugea bombycis*	Kudo	(see 1916)
1912	*Nosema bombycis*	Omori	1912 : 108-122
1913	*Nosema bombycis*	Kudo	1913 : 368-371
1916	*Nosema bombycis*	Kudo	1916 : 31-51
1918	*Nosema bombycis*	Kudo	1918 : 141-147

Habitat: In every tissue of eggs, larvae, pupae and imagos of *Bombyx mori*. Stempell succeeded in causing experimental infection in the larvae of *Arctia caja*.

Locality: Cosmopolitan in occurrence: France, Italy, Germany, Austria, Hungary, Nippon, India.

The nature of parasitism. The parasite causes a chronic disease in silkworms which is known under various names such as pébrine, maladie des petits, etisie, maladie corpusculeuse (France), gattina, malattia dei corpuscoli, mal delle petecchie (Italy), Körperchenkrankheit, Fleckenkrankheit (Germany), Cota (India) and Kokushibio, Biriushibio (Nippon). When left unchecked, the disease under favorable conditions spreads among the host larvae and to the offspring through the ova, assuming an epidemic form. The outbreak in the middle of the last century of the epidemic in the sericultural countries of Europe is well known, and led to studies and observations of an enormous number of investigators on that continent and later in other parts of the world, on the nature, structure and life-history of the microsporidian and also on measures for controlling the disease.

Symptoms of diseased insects. When a group of worms hatched out at the same time, is infected by the parasite, irregular growth is to be noted usually after the first or second moult, some growing normally, while many diminish in size after attaining a certain size. Although lightly infected larvae do not show any particular symptoms, those heavily infected show some abnormalities. The latter move about sluggishly, lose their appetite, grow very slowly or not at all, and succumb to death before pupation. They may be able to spin cocoons which are, however, very thin and poor in quality. The silk threads of such cocoons are very irregular in thickness and very weak so that they break easily during the processes of reeling. The most characteristic symptoms of the advanced stage of the disease are the dark brown or black spots with irregular but distinct contours, which appear over the surface of the host body especially on its posterior ventral side. These spots are generally well visible on the larvae at the fourth or fifth stage (Fig. 757). In pupae and moths these spots are found on various parts of the body. Infected moths show very often unexpanded antennae or wings. As to the formation of the black spots mentioned here, Sasaki (1897) thought that hemorrhage through the wounds in the integument, produced them by the coagulation of the blood. Stempell (1909) states that when a hypodermal cell is attacked by the microsporidian, the chitin-cuticula over the cell becomes brownish in color, and brittle, disintegrating into several fragments, and forming a space into which the cell filled with the spores drops. The remaining hypodermal cells regenerate and form a continuous layer. When the epithelial cells secrete a new layer of chitinous substance, the spores enclosed in the space become yellowish in color due to the lack of oxygen and produce a spot which appears dark to the naked eye.

The organs of the more or less heavily infected individual exhibit a milky-white appearance due to the presence of a large number of spores. This state is especially noticeable in the silkglands where the epithelial cells become distended, forming irregular tumor-like pustules of white color in contrast with the translucent normal cells.

Pasteur, Bolle and others believed that the Nipponese breeds of *Bombyx mori* have stronger resistance against the microsporidian than any other breeds. That is to say, they spin cocoons, although they are infected. In Nippon, a similar view is held by the practical silk-worm breeders and the results obtained in the Imperial Sericultural Station seem to support this view.

Modes of infection. The microsporidian enters the host larvae either through the eggs while the latter develop in the infected ovaries of the female moth or through the digestive tract when taken into it with the food. The soft fecal matters of the infected host larvae are, of course, the most dangerous source of infection, since the spores contained in them are easily spread over the mulberry leaves on which the worms feed usually in a very crowded condition.

Preventive measures. The sources of infection have to be eliminated. Infected eggs and other stages of the host insect must be destroyed as soon as possible by frequent and careful inspection. Pasteur invented "grainage cellulaire" by which the moths are separated from one another in pairs. After oviposition, they are examined separately under a microscope and if they contain the spores of the microsporidian, the batches of eggs laid by them are destroyed. This process would, of course, eliminate the infection from the moth to the next generation. To exclude the other source of infection, diseased worms must be destroyed as soon as possible. This will be done by general inspection of the growth of the larvae and the symptoms of the disease. The spores are highly resistant to chemicals because of the tough membrane. Vast numbers of chemicals were recommended by many authors, among which formaldehyde vapor seems to be the most effective. Extreme high temperature and humidity tend to accelerate the growth of the parasite in the host body and to weaken the resistant power of the host larvae against the development of the parasite.

Vegetative form. Balbiani (1884) thought the development of the microsporidian was as follows: The infection takes place when silk-worm larvae swallow the spores. By feeding healthy young larvae on mulberry leaves smeared with the spores, they become infected in a few days. The alimentary canal was drawn out and examined microscopically. Spores were first recognized in the stomach, later in the epithelial cells or muscular tissue surrounding the gut. In the alimentary canal of the host, the spore opens at one end and the amoeboid sporoplasm creeps out and enters various organs. The actual penetration of the parasite through the lining epithelium could not be observed. The amoeboid body grows up in the tissue, its nucleus multiplies by division and the contents break up into numerous spherical bodies, each of which develops into a single spore.

Pfeiffer (1888) confirmed Balbiani's observations and mentioned the changes of the spores which he noted in the hanging crop preparations as follows: When spores tkane from the dead and dried moths are mixed with peptone-meat-extract, with the blood of the normal larvae, or with the cow's serum in hanging drops, and are kept in an incubator at 20°C, some spores show after 24 hours dark spots or small rounded or angular bodies which are connected with the spores by short threads. In other instances an amoeboid body was noticed to leave the spore. In from 24 to 36 hours, a large number of free amoebulae were seen in active movements. Similar changes were noticed in the stomach of *Gryllus campestris* when infected through mouth. Léger and Hesse (1907) mentioned that the sporoblast develops into a single spore.

Stempell's (1909) observations may be summarized as follows: The parasite starts its life cycle in the host body with the stage that the author termed planonts. A planont is a direct product of the sporoplasm of the spore which entered into the stomach with the food, being found in the foregut and midgut on the first and second days after artificial infection. The planont is found in extracellular state and is a small body of 0.5 to 1.5μ in diameter (Figs. 1, 2). In permanent preparations, it is usually a deeply stained spherical body with a granular nucleus surrounded by a clear space. By the appearance and size, it is distinguished from the cocci and chrolophyll grains which coexist in the gut. The planonts multiply actively by binary fission or budding, and thus are usually found in large groups They apparently have amoeboid movements, as they are found in the intercellular space between the epithelial cells of the gut, although the movements were not actually observed. They migrate into the blood, and with its movements reach various parts of the host body. New infection takes place in the hemocoel and then in host cells. The epithelial cells of the alimentary canal are infected in the majority of cases by planonts which enter them from the base of the cells. The planonts probably invade the ovum. In an experimental host larva, a large number of planonts and relatively small number of meronts and spores were found on the fifth day after the infection experiment started. Stempell thinks that this may be due to immunity in which the host tissues showed resistance against the invasion of the planonts and further intracellular development of the microsporidian.

When the planont penetrates through the host cell wall and enters the cytoplasm, it becomes a meront (Fig. 3). The meronts appear on the second day of infection and succeeding days and multiply actively especially in the epithelial cells of the midgut. The meront, about 3 to 5μ in diameter, has a pellicula-like external layer which is well seen in the dividing form. Its cytoplasm of fine reticular structure, stains less deeply than that of the planont. The external layer prevents the formation of a pseudopod, but does not hinder the absorption of nutrition, since the meront secretes a peptonizing ferment which dissolves the surrounding cytoplasm of the host cell. Therefore the meronts are always found in fluid vacuoles in the host cell. The meront possesses a nucleus. It is relatively large nucleus composed of number of chromatic grains. The meronts multiply according to one of the three types of divisions: binary fission, budding or multiple division. Binary fission which is the commonest, is completed in a comparatively short time, so that the changes can be traced in living specimens. The nucleus divides amitotically. The connection between the daughter nuclei often remains in arch form (Figs. 4-7). The cytoplasmic constriction is frequently not complete after the nuclear divisions, thus producing a chain form or sausage form with the nuclei up to seven in number (Figs. 8, 9), although the former does not occur so often as in *Thelohania mülleri*. The daughter meronts often remain round without elongation of the body. The size is frequently unequal, and hence Stempell maintained this as budding (Figs. 10-12). Further the meront often simply forms a partition and then another in the interior without any external changes, thus forming a tetranucleate rounded body (Figs. 13, 14). This is one phase of multiple divisions. As to the occurrence of these various modes of multiplication, Stempell believes that the microsporidian which invades different tissue cells of the host, adapted itself to the environment to increase its rate of multiplication. Where the multiplication direction is unlimited, spherical binary fission takes place; in the tissue such as the muscular tissue, parallel chain formation takes place; while where a little space is left for the daughter meronts, partition division and multiple division take place Rarely abnormal meronts are found, which are large and produce spores by endogenous budding. The meront does not invade the cell membrane or the nucleus of the host cell, although it occupies the entire cytoplasm of the host cell. In the egg-cell, the meronts are found in the center and apparently do not disturb the development of the embryo. Some of the meronts later become spores, while others go through a resting stage which apparently coincides with that of the embryo, after which they multiply and enter directly the embryonic cells. If a spore enters the gut of the embryo, it undergoes exactly the same changes as it would if reached the gut of a host larva.

When the lack of nutrition or space appears, the meront becomes transformed directly into a single spore. The spore membrane begins to appear around it on the third day and is completed on the fourth day of infection. The cytoplasm condenses and leaves fluid vacuoles which unite into a large one at one end of the spore (Textfig. D, q). The cytoplasm assumes a girdle shape in the middle of the body which now becomes oval in shape (r). The nucleus becomes lodged at one end and divides into four by two successive binary fissions; these nuclei are at first of the same size, but later show different dimensions. Usually the nucleus seems to bud off a small body which in turn divides into two small nuclei that probably control the membrane formation (q). The large nucleus buds off another small one which becomes situated in the region of the main mass of the cytoplasm to form the polar capsule (q to s). These three nuclei become invisible when the spore membrane and the capsule are completely formed. The remaining nucleus divides into two of equal size and remain in the cytoplasm (s). While the membrane is still thin, the spore appears pyriform due to the presence of a large vacuole at one of the ends. (Fig. 18) Later as the membrane grows thicker, the spore shows its typical oval form (Fig. 17). As the refractivity of the spore membrane is equal to that of Canada balsam the spore appears to be much smaller in this medium than in any others with smaller refractivity. Abnormalities of the spores are solely due to those of the meronts. The entire life-cycle from spore to spore, is completed in four days under favorable circumstances.

Omori (1912) observed that the simplest stage was a uninucleate or binucleate round or irregular body, found in the muscle fibers of the gut. With Giemsa, the cytoplasm and the nucleus stains blue and red respectively as usual. The body increases in size and the number of nuclei to eight, which are arranged in four pairs. The body breaks up into binucleate daughter cells, later into uniucleate cells. Budding or multiple division observed by Stempell was not seen. The schizont develops into a single spore. The young spore has a single nucleus and a vacuole at one end.

Kudo (1916) states that the youngest form found in the gut of the host is a small mass of protoplasm containing two nuclei (Fig. 21). It is 1 to 1.5μ in diameter and of oblong or triangular form. With Giemsa, the cytoplasm stains blue or feebly red, while the nuclei take a deep red color. It has most probably amoeboid movements, judged by its irregular form. The so-called planont stage described by Stempell was not recognized. The nuclei of the amoebula fuse into one. The next stage was found in the gut-epithelium, fat body, etc. The schizont (Fig. 22) is 1.5 to 2.5μ in diameter. Its cytoplasm is dense and stains deeply. The nucleus is a rounded compact granule with a diameter of 0.5μ. Stained with Giemsa, the cytoplasm takes a blue color, while the nucleus deep red. The schizont multiplies by binary fission (Figs. 23-26) or multiple division (Figs. 27,28.) The nuclear division is of the amitotic type. Chain formations were frequently observed in the epithelial cells of the infected embryo. Multiple division of the schizont into three is as common as that into four individuals. The schizont develops into a single spore. The two nuclei of the schizont fuse into one, thus forming a uninucleated sporoblast. The cytoplasm condenses toward the center in a girdle-shaped mass, leaving a vacuole at one end. The nucleus divides amitotically: one of the nuclei divides again into two. One of the latter two divides into two. As a result there are formed four nuclei of different size

(Fig. 31); the large one is the sporoplasmic nucleus which later divides into two, the medium-sized one is the capsular and the other two are parietal nuclei which form the spore membrane.

As to the propagation of the parasite in the host body, Kudo is of the opinion, contrary to Stempell, that the spore whether taken into the gut with the food or formed in the same host individual, germinates under the influence of the digestive fluid.

Spore: Balbiani (1884) described the spore as covered with a smooth and structureless membrane which is not bivalve. At one end a vacuole is often seen, while the nucleus is hardly noticeable. The spore is highly resistant against chemicals. Dimensions: length 4μ, breadth 2μ. Thélohan (1894) detected the polar filament in the spore of this species and gave its dimensions (1895) as follows: length 3μ, breadth 1.5 to 2μ; when treated with nitric acid, length 6μ, breadth 3 to 3.5μ, length of polar filament 10 to 15μ. Léger and Hesse (1907) observed two valve cells, a capsulogenous cell with a small nucleus and a sporoplasm with one or two nuclei in the spores.

Stempell (1909): Pyriform, in young spores (Fig. 18) or oval, in mature spores (Figs. 17, 19, 20). A vacuole is seen at each end (Fig. 16). The girdle-shaped sporoplasm has two nuclei which divide into four when the spore enters the gut of a new host (Textfig. D, s to u). Spore membrane 0.5μ thick. Dimensions of spores vary, which has no other meaning than abnormality. The presence of capsule and filament is difficult to prove. Some spores, however, show frequently a dark area (Fig. 19) when treated with iodine alcohol or nitric acid and seen with obliquely directed light. Stempell by means of microphotography with ultraviolet rays, noticed the coiled polar filament in the plates; the figures, however, are unfortunately not clear enough to support his statement. The filament according to Stempell is about 0.1μ thick and the capsule is about 2μ by 1μ. By calculation he held that the filament is coiled in the capsule about ten times and that the filament runs from the anterior end toward the opposite end, the remaining portion being coiled around the axis. Some spores are seen to extrude the filaments in the gut of the new host on the second day of infection (in some cases even after six hours only). According to Stempell, extrusion of the filament and emergence of the sporoplasm probably take place under the influence of the saliva, since if the digestive fluid of the midgut has this effect, the spores formed in and liberated from the epithelial cells of the midgut would germinate in the same host. Under the action of the digestive fluid the sporoplasm in the spore becomes enlarged and its pressure upon the capsule probably causes extrusion of the filament. The filament serves for temporary fixation of the spore near the gut-epithelium. Through the opening made by filament extrusion, the sporoplasm creeps out by amoeboid movements, but this process, however, was not actually observed.

Two of the four nuclei of the sporoplasm are left behind when the latter leaves the spore membrane as a binucleate amoebula. The fusion of the two nuclei most probably takes place. Dimensions of the spore: length 4μ, breadth 2μ, length of polar filament 32 to 34μ, abnormal spores reach 6μ by 4μ, 20μ by 10μ.

Omori (1912): elongated, often somewhat beanshaped, highly refractive. The structure could not be studied and Omori thought it similar to that of *Plistophora longifilis* described by Schuberg in whose laboratories he worked. Dimensions: 2 to 4μ, breadth 1 to 2μ. Nothing is mentioned about the filament.

Kudo (1913, 1916, 1918): Oval. The form and size are variable to a certain extent. No definite dimorphism was noticed. The spore membrane appears to be thinner than given by Stempell. Giemsa's stain penetrates with little difficulty. The girdle-shaped sporoplasm is clearly a ring form in optical cross section. The sporoplasm has usually two nuclei in the fully grown spore (Figs. 32, 33) and one nucleus in young spore (Fig. 31). The polar capsule is brought into clear view by treating the spore with nitric acid as Thélohan did (Figs. 34-36). It is ellipsoidal and connected with the anterior end of the spore. The filament of a fresh spore is easily and instantaneously extruded under the action of perhydrol or mechanical pressure (Fig. 39), while dessicated fresh spores extrude their filaments under mechanical pressure. A spore emulsion centrifuged with 60 per cent. methyl alcohol for ten minutes or mixed with 34 per cent. ethyl alcohol for 16 hours shows an extruded filament when treated with perhydrol. The filament seems to be coiled without a central axis (as in *Stempellia magna*). The extruded filaments are well stained with Löffler's or Fontana's staining and can be made into permanent preparations.

By mixing the fresh spores with a drop of the digestive fluid of normal host larvae on a slide and keeping it at room temperature for 24 hours, Kudo noticed that the sporoplasms of some spores moved toward the anterior end in a round or irregular form and caused the extrusion of the filaments (Figs. 37, 38). Further observations could not be made. Artificial cultivation *in vitro* failed. Dimensions of spores: length 3 to 4μ, breadth 1.5 to 2μ, polar capsule, 1.5 to 2μ by 0.8 to 1μ, length of extruded filament 57 to 72μ, or even up to 98μ.

NOSEMA PARVA Moniez 1887

1887	*Nosema parva*	Moniez	1887a : 1313
1895	*Glugea leydigii*	Pfeiffer	1895 : 83, 86

Habitat: *Cyclops* spp.

Locality: France (Lille) and Germany (Weimar).

Vegetative form: Moniez simply states that the sporogenic masses are relatively voluminous. Pfeiffer describes the "cysts" with the spores are rounded or elongated. The so-called cyst with large spores is about half the size of that with the smaller spores.

Spore. Moniez: Oval, with a clear space regularly at one end. Size, 3.5μ by 2μ. After Pfeiffer: pyriform, with a clear spot at the rounded end. Dimensions: 8μ by 5μ.

Remarks: The generic designation is doubtful. Moreover the identity of the two forms is also open to question because of the unusual difference in size of the spores between Moniez's and Pfeiffer's forms. The original generic name is kept here provisionally.

NOSEMA BRYOZOIDES (Korotneff 1892) Labbé 1899

[Figs. 40-60]

1892	*Myxosporidium bryozoides*	Korotneff	1892 : 591-596
1895	*Glugea bryozoides*	Thélohan	1895 : 359
1899	*Nosema bryozoides*	Labbé	1899 : 106
1911	*Nosema bryozoides*	Braem	1911 : 19-29
1914	*Nosema bryozoides*	Schröder	1914 : 320-323

Habitat: Testicular cells and body cavity of *Plumatella* (*Alcyonella*) *fungosa* and *P. repeus*.

Schröder noticed that the nuclei of the infected host cells became hypertrophied and divided amitotically.

Locality: Turkestan (Issyk-Kul), Russia (Moskau, May to August), Germany (Schleswig-Holstein and southern Germany).

Vegetative form: Korotneff (1892): Small spherical bodies attached to the funiculus (Fig. 40). As the development of the reproductive organ of the host progresses they appear about the end of May. At first they are microscopical, but in July they are recognized with the unaided eye. When the infection becomes intensive in August, the host body-cavity is filled with these bodies. The amoeboid forms ("Myxosporidien Klumpfen") vary in diameter from 20μ to 200μ. Small forms are spherical, while larger ones are oval or lobose. The cytoplasm is differentiated into ectoplasm and endoplasm. The ectoplasm differentiates fine pseudopodia (Fig. 41, similar to those of *Myxidium lieberkühni*, a myxosporidian, observed by Bütschli). Young amoebae are attached to the funiculus by means of the pseudopodia.

Braem (1911): The microsporidian was found in cystid, polypid and so-called embryo. Its occurrence is limited to the testicular cells which are attached to the funiculus (Fig. 47). The parasites later float around in the body cavity after becoming detached from the funiculus (Figs. 43, 44, 57), and finally go to pieces setting free the spores. No early stage of the infection was observed. The microsporidian attacks the spermatogonia (Fig. 46) and the host nucleus undergoes degeneration. Large oval bodies containing mature spores are conspicuously seen attached to the funiculus; these correspond to the spermatospheres. These bodies fall into the body cavity and are found in groups among polypides. They are of round form with a diameter of 40μ or more. The largest one observed was 130μ by 70μ. The cytoplasm is distinctly differentiated into ectoplasm and endoplasm, the former forming pseudopodia (Fig. 57) and the latter producing spores, sporoblasts, and nuclei which vary from less than 5μ to 9μ in diameter.

Schröder (1914): The microsporidia penetrate through the cell membrane of the testis and become schizonts (meronts). They divide repeatedly and multiply in number. When unfavorable conditions appear, the schizonts develop into sporonts each of which becomes in turn a spore. When the parasites occur in large numbers, the infected cell assumes an oval form in which one sees schizonts, sporonts, spores, and more or less changed host nuclei. The oval body becomes isolated from the funiculus and finally falls into the host body-cavity (Fig. 58); this oval body is formed by the fusion of numerous spermatogonia. It is of sausage form and has the dimensions of 40 to 50μ by 30μ. It is not a parasitic body as was thought by Korotneff and Braem, but an infected host cell. Schröder did not observe any structure which might be called a pseudopodium as seen by Korotneff and Braem, and maintain that the so-called pseudopodia seen by the latter two authors are in reality the differentiated cytoplasm of the host cell. The schizonts are spherical to ellipsoidal, each having a single nucleus. Chain formation by schizogony apparently does not occur.

Spore. Korotneff: Elongated ovoidal; one end attenuated, the other rounded (Fig. 42). Highly refractive in fresh condition. Some show two vacuoles at the extremities. A refractile granule at the pointed end is the polar capsule (?). Dimensions (measured from Korotneff's figures): length 10μ, breadth 6μ. Thélohan gave similar dimensions in his monograph.

Braem: Oval, one end attenuated (probably more attenuated than is shown in figure). In preserved material, such a sharp point as figured by Korotneff was not recognized. Shell is double-contoured. Young spores have always two large deeply staining nuclei which resemble the semicircular nuclei of the young stages. Dimensions: length 7 to 8μ, breadth 5 to 6μ.

Schröder: Ellipsoidal (Figs. 59, 60). Mature spores with slightly attenuated anterior end; circular in transverse section. The polar filament was extruded from some spores when treated with hot concentrated nitric acid. Dimensions: length 7μ, breadth 4μ, rarely 10μ by 5μ, length of filament 30 to 40μ.

NOSEMA CILIATA (Mrázek 1897) Kudo

[Figs. 61-63]

1897	*Myxocystis ciliata*	Mrázek	1897 : 1-5
1910	Myxocystis	Mrázek	1910 : 245-258

Habitat: Lymphocytes of *Limnodrilus claparedianus*. The microsporidian is very rare, being found only in a single host (in the spring, 1896), although "many thousands of different Tubificidae" were examined. The infected host animal was greyish yellow in color so that it was easily distinguished from variously colored normal animals. This change in coloration is due to the presence of infected lymphocytes. In his first paper, Mrázek thought that the infected host cell was the parasite, hence the genus Myxocystis.

Locality: Czechoslovakia.

Vegetative form: This description is taken from Mrázek's two papers. The infected lymphocytes vary from 50 to 100μ in diameter. They are spherical or ellipsoidal in form (Fig. 61) and frequently possess short and fine projections (Fig. 62) which Mrázek first compared with pseudopodia of the trophozoites of some Myxosporidia. The nuclei of the host cell vary in number and form, being usually large in number and irregular in form in greatly infected lymphocytes. Schizogony and sporogony are not described.

Spore: Oval (Fig. 63). Length 4μ. The spore possesses one or two refractive granules in it.

NOSEMA MARIONIS (Thélohan 1895) Stempell 1919
[Figs. 64-71]

1895	*Glugea marionis*	Thélohan	1895 : 360
1909	*Mariona marionis*	Stempell	1909 : 341
1917	*Glugea marionis*	Georgévitch	1917 : 1, 10-11 1917a : 106-107
1919	*Nosema marionis*	Stempell	1919 : 114, 142-144

Habitat: In the cytoplasm of the trophozoites of *Ceratomyxa coris*, a myxosporidian, parasitic in the gall-bladder of *Coris julis* (*Julis vulgaris*) and *C. giofredi* (*J. giofredi*). This microsporidian is a very interesting form in that it is the only recorded species of Microsporidia, which is parasitic in a myxosporidian. At the time of his discovery of this species, Thélohan (1895) did not see the spores of the myxosporidian to which Georgévitch (1917) gave the name, *Ceratomyxa coris*, and therefore Thélohan held it as a species of Glugea of an exceptional character.

Georgévitch observed the myxosporidian more frequently than the microsporidian and wondered why Thélohan failed to see the former spores. The sporulation of these two forms of Cnidosporidia took place in many cases in one and the same plasmodium. Georgévitch interpreted it as the result of a plasmogamy of the two kinds of trophozoites. In my monograph on Myxosporidia (Kudo 1920a) I remarked that "Georgévitch (1917: Fig. 30) observed that spores of *Glugea marionis* occurred in disporous trophozoite of *Ceratomyxa coris*, which he thought happened accidentally by plasmogamy of these two Cnidosporidia.The above mentioned figure, however, strongly suggests that *G. marionis* may be leading a parasitic life in the trophozoite of *C. coris*." This supposition was justified by Stempell's (1919) delayed paper which did not reach me until the fall of 1920, due to the post war condition.

Stempell (1919) noticed that out of 36 fish infected by *Ceratomyxa coris*, 15 showed the myxosporidian infected by *Nosema marionis*. Stempell thinks that the microsporidian spores enter the gall-bladder of the fish through the alimentary canal and infect the trophozoite of the myxosporidian. By the division of the infected myxosporidian, the number of infected individuals is increased. The author thinks that the Nosema spores would most probably not germinate in the gall-bladder in which they were formed.

Locality: France (Marseille, Villefranche).

Vegetative form: Thélohan's description was that of the trophozoite of *Ceratomyxa coris* (Fig. 64). Georgévitch does not add any information on this phase.

Stempell (1919): The development is exactly similar to that of *Nosema bombycis* he studied. The youngest form (planont) is intracellular and possesses a large nucleus. It becomes a meront (Fig. 66). The nuclear division of the meront is promitosis. Each sporont develops into a spore. Chain form of meronts was not seen, probably due to the movements of the myxosporidian trophozoite; nuclear divisions are not frequently followed by cytoplasmic constriction and commonly a multinucleate form is produced. In some host trophozoites one may see 50 or more spores (Fig. 67). The spore formation seems to be carried on in a way similar to that of *N. bombycis*. Nuclear divisions are noticed occasionally and there are to be seen seven nuclei in the developing spore.

Spore. Thélohan: Highly elongated ovoidal (Fig. 65); slightly attenuated. Dimensions: Length 8μ, breadth 3μ. Georgévitch: structure (Figs. 68 to 71) similar to that of *Plistophora macrospora* as described by Léger and Hesse (1916). Some spores lack the polar capsule completely (Fig. 71).

Stempell (1919): Form elongated oval, with or without a clear rounded space at one end. Coiled polar filament is rarely visible in fresh spores. Dimensions: 1.5 to 7μ. Treating the fresh spores with iodine water did not cause the filament extrusion.

NOSEMA LOPHII (Doflein 1898) Pace 1908

[Figs. 72-84, 758]

1898	*Glugea lophii*	Doflein	1898 : 290, 332-338
1899	*Glugea lophii*	Mrázek	1899 : 1-8
1901	*Glugea lophii*	Scott	1901 : 343
1908	*Nosema lophii*	Pace	1908 : 67-70
1911	*Glugea lophii*	Weissenberg	1911 : 149-157 1911a : 383-421
1911	*Nosema lophii*	Weissenburg	1911b : 344-350

Habitat: Central nervous system of *Lophius piscatoris* and *L. budegassa spinola.* The microsporidian produces large and conspicuous tumors in the host system (Fig. 758), which are composed of cysts of the microsporidian according to Doflein and Pace, or of hypertrophied ganglion cells according to Mrázek and Weissenberg. Weissenberg studied the organism at Naples in March and May. In 1909, he found 14 infected fish out of 21 examined, while in 1910, 7 were found to be infected among 22 fish examined.

Locality: Austria (Rovigno, Triest), Italy (Naples), France (Le Croisic), England (Liverpool Bay).

Vegetative form: Doflein (1898): Cysts are very large and conspicuous (Fig. 758) and show milky white masses in the fresh state. They contain fatty substances which decrease in quantity as the spores develop. The tumors which are composed of groups of cysts are of irregular form and present grape-like appearances. Each pansporoblast contains spores more than ten in number.

Mrázek (1899): The cyst lies in the processes of the ganglion cell, but not directly in the cell. The infected ganglion cell becomes extremely hypertrophied; by growth of the connective tissue around the cell and the cyst, the nerve fiber bundles become separated from one another. The nucleus of the host cell becomes large and multilobated, being poor in chromatic substances. The fibrous structure of the host cell body becomes more and more pronounced, until the cytoplasm of the cell and its processes changes into a somewhat loose fibrous network. In sections, the cyst is differentiated into two portions, a pale inner and deeply stained outer regions, which distinction is due to the difference in the ground substance between these two parts. The outer region branches into the interior part of the cyst, forming irregular chambers. The cyst is filled with spores. Leucocytes take the spores in (Fig. 78).

Pace: The tumor is composed of densely placed cysts. Usually the cyst is single, sometimes two or three, however, fuse into one. In young cysts stained after Mann's method, four zones are to be distinguished: an external zone filled with loose fibers and pansporoblasts, a zone of clear blue color with numerous nuclei and spores of the microsporidian, a central zone with spores stained red with eosin and an intermediate zone between the second and third.

Weissenberg: The infected ganglion cell shows no Nissl's granule nor neurofibrilles, but exhibits fine thread-like structure which can be stained with an ordinary method. These ganglion cells are much larger than the normal ones. In a 23 cm. long fish, the youngest cyst found was 300μ in diameter, while the largest normal ganglion cell was 100μ in diameter. With the growth of the cyst, the ganglion cell, both cytoplasm and nucleus with its nucleolus, become hypertrophied to an immense extent. In sections, three parts are distinguished in the cyst; i. e., spores, vegetative forms, and ground substance. In cysts of medium size, there are regularly two zones of spores, the outer and the inner. The outer zone contains spores of oval form, while in the inner zone the ground substance is more fluid and the spores are more cylindrical. The oval spores most probably develop into the cylindrical form. These two zones show different aspects by staining due to the different size of the nuclei and to different affinity of the spores toward the staining reagents. Osmium fixation gives deep brown coloring of the outer zone and light yellowish staining of the inner region. The ground substance of the cyst is homogeneous. No typical cyst membrane exists, although the outer surface takes the stain intensively. The ground substance is most probably the cytoplasm of the host cell which becomes changed by the parasitism and assumes a homogeneous appearance.

Schizonts (Fig. 79) are observed in young cysts. They are polygonal to rounded in form and are stained deeply in contrast with the ground substance which stains deeply with iron haematoxylin. They contain a nucleus. Elongated forms with two nuclei are often seen (Fig. 80). These are apparently dividing forms. As the cytoplasmic division does not always follow a nuclear division, chain-forms (Fig. 81) are produced. The schizonts transform themselves into sporonts each of which becomes a spore. Hence the development is similar to that of the genus Nosema. Thus the change of the species from the genus Glugea to Nosema as advocated by Weissenberg is justified.

Spore. Doflein: Oval, often curved in bean shape (Fig. 74). The polymorphism of the spores is due to the pressure upon them while still in the pansporoblasts. The polar capsule is present; filament extrusion, however, was not observed. Dimensions: length 3.5μ, breadth 1.5μ.

Weissenberg: Spores are of two types and of various size. Oval spores occur in the peripheral region and cylindrical spores occur in the central portion of the cyst. When stained with iron hematoxylin, the sporoplasm appears as a girdle-shaped mass. The spores possess two vacuoles, one at each extremity; one is always clear, while the other appears sometimes dark or greyish in color. The clear vacuole is larger in the oval spore, and is smaller in the cylindrical spore than the one which stains dark. In the dark vacuole, the filament is probably coiled. The nucleus could not be studied owing to the minuteness of the object. In younger cyst, oval spores (Fig. 82) are mostly found, while in older one, cylindrical spores (Figs. 83, 84) are to be seen in a large number. The significance of the two types of spores is unknown to Weissenberg.

NOSEMA VANILLAE *a* Lutz et Splendore 1903

[Fig. 85]

1903	*Nosema vanillae a*	Lutz and Splendore	1903 : 154, 155

Habitat: *Dione vanillae* (Lepidoptera). The authors did not state the location in the host of this and other species which are listed here below except that in the Lepidoptera, the seat of spore formation was in the intestine, Malpighian tubules, silk glands and reproductive glands. In the intestine the epithelium, muscle and tracheae are infected. When the infection is common, the fat body and muscles in other parts of the host become involved. Isolated spores are to be seen throughout the body so that the infection of a moth is diagnosed by microscopical examination of its wing.

Locality: Brazil.

Spore: Ovoidal; one end is broader than the other. Bilaterally symmetrical. The largest breadth coincides with the equatorial plane. Dimensions: length 2.5 to 2.75μ, breadth 0.85 to 1.3μ.

Remarks: Lutz and Splendore (1903, 1904, 1908) described a number of Microsporidia under the genus Nosema. Since sporogony was not studied, the generic designation is open to question. They are therefore listed here provisionally in this genus.

NOSEMA VANILLAE β Lutz et Splendore 1903

[Fig. 86]

1903 *Nosema vanillae β* Lutz and Splendore 1903 : 154, 155

Habitat: *Dione vanillae.* More frequently encountered than *α* form.

Locality: Brazil.

Spore: More or less elongated oval or cylindrical. Length 2.5 to 3.5μ, breadth 1 to 2μ.

NOSEMA VANILLAE γ Lutz et Splendore 1903

[Fig. 87]

1903 *Nosema vanillae γ* Lutz and Splendore 1903 : 154, 155

Habitat: Same as before.

Locality: Brazil.

Spore: Elongated cylindrical, arched. Length 3.5 to 6μ, breadth 2 to 3μ.

NOSEMA ASTYRAE Lutz et Splendore 1903

[Fig. 88]

1903 *Nosema astyrae* Lutz and Splendore 1903 : 154, 155

Habitat: *Brassolis astyra* (Lepidoptera). Highly infected pupae died rapidly.

Locality: Brazil.

Spore: Ovoidal. Length 4 to 4.5μ, breadth 2.5 to 3μ.

NOSEMA GIRARDINI Lutz et Splendore 1903

[Fig. 89]

1903	*Nosema girardini*	Lutz and Splendore	1903 : 154, 155

Habitat: *Girardinus caudimaculatus* (fish, Cyprinodontidae). In the integument, muscles and serosa and mucosa of intestine.

Locality: Brazil.

Spore: Pyriform. Length 2 to 2.5μ, largest breadth 1 to 1.5μ.

NOSEMA JUNONIS α Lutz et Splendore 1903

[Fig. 90]

1903	*Nosema junonis*	Lutz and Splendore	1903 : 154, 155
1904	*Nosema junonis α*	Lutz and Splendore	1904 : 645

Habitat: *Dione juno* (Lepidoptera). The authors infected experimentally the larvae of *Papilis pompejus* with the microsporidian.

Locality: Brazil.

Spore: Oval to elongated ovo-cylindrical. Length 3.5 to 8μ, breadth 1 to 2μ. The infection was seen frequently, but the infected larvae ordinarily metamorphosed completely.

NOSEMA JUNONIS β Lutz et Splendore 1904

[Fig. 91]

1904	*Nosema junonis β*	Lutz and Splendore	1904 : 645

Habitat: *Dione juno.*

Locality: Brazil.

Spore: Somewhat regularly oval. Length 3.5 to 4.5μ, breadth 1.7 to 2μ.

NOSEMA LYSIMNIAE Lutz et Splendore 1903

[Fig. 92]

1903	*Nosema lysimniae*	Lutz and Splendore	1903 : 154, 155

Habitat: *Mechanites lysimnia* (Lepidoptera).

Locality: Brazil.

Spore: Oval or pyriform. Length 4 to 6μ, breadth 2 to 2.5μ.

NOSEMA EUBULES Lutz et Splendore 1903

[Fig. 93]

1903	*Nosema eubules*	Lutz and Splendore	1903 : 154, 155

Habitat: *Catopsilia eubule* (Lepidoptera).

Locality: Brazil.

Spore: Oval, pyriform or cylindrical. Length 2 to 5μ, breadth 1 to 2.5μ.

NOSEMA LOPHOCAMPAE Lutz et Splendore 1903

[Fig. 94]

1903	*Nosema lophocampae*	Lutz and Splendore	1903 : 154, 155
1904	*Nosema lophocampae*	Lutz and Splendore	1904 : 649

Habitat: *Lophocampa flavostica* (Lepidoptera).
Locality: Brazil.
Spore: Cylindrical, bent. Length 3.5 to 4μ, breadth 1 to 2μ.

NOSEMA ERIPPI Lutz et Splendore 1903

[Fig. 95]

1903	*Nosema erippi*	Lutz and Splendore	1903 : 154, 155

Habitat: *Danais erippus* (Lepidoptera) and probably *D. gilippus.*
Locality: Brazil.
Spore: Irregular oval or ovo-cylindrical. Length 3 to 3.5μ, breadth 1.5 to 2.5μ.

NOSEMA HELIOTIDIS Lutz et Splendore 1904

1904	*Nosema heliotidis*	Lutz and Splendore	1904 : 645

Habitat: *Heliotis armigera* (Lepidoptera).
Locality: Brazil.
Spore: More or less elongated oval. Length 2.5 to 5.5μ, breadth 1.7 to 2μ.

NOSEMA HALESIDOTIDIS Lutz et Splendore 1904

1904	*Nosema halesidotidis*	Lutz and Splendore	1904 : 648

Habitat: *Halesidotis* sp. (Lepidoptera).
Locality: Brazil.
Spore: Similar to *Nosema lophocampae,* but undetermined.

NOSEMA CAECULIAE Lutz et Splendore 1904

[Fig. 96]

1904	*Nosema caeculiae*	Lutz and Splendore	1904 : 646

Habitat: *Caeculia* spp. (2) (Lepidoptera).
Locality: Brazil.
Spore: Regularly elongated oval, often with a vacuole. Length 5 to 6μ, breadth 2 to 2.5μ.

NOSEMA HYDRIAE *a* Lutz et Splendore 1904

[Fig. 97]

1904	*Nosema hydriae*	Lutz and Splendore	1904 : 646

Habitat: *Hydria* sp. (Lepidoptera).
Locality: Brazil (Petropolis).
Spore: Bacilliform. Slightly flattened along the entire length so that in cross-section it is ellipsoidal. Length 4 to 5.5μ, breadth 1 to 1.5μ.

NOSEMA HYDRIAE β Lutz et Splendore 1908

1908 *Nosema hydriae β* Lutz and Splendore 1908 : 314-315

Habitat: *Hydria* sp.
Locality: Brazil (Amazon district).
Spore: Regularly ovoidal or ovocylindrical; a distinct round vacuole at rounded end. Length 3.5 to 5.5μ, breadth 2 to 3μ.

NOSEMA HYDRIAE γ Lutz et Splendore 1908

1908 *Nosema hydriae γ* Lutz and Splendore 1908 : 315

Habitat: *Hydria* sp.
Locality: Brazil (Amazon district).
Spore: Somewhat smaller than β form.

NOSEMA MICRATTACI Lutz et Splendore 1904

[Fig. 98]

1904 *Nosema micrattaci* Lutz and Splendore 1904 : 646

Habitat: *Micrattacus nanus* (Lepidoptera).
Locality: Brazil.
Spore: Regularly oval or ovocylindrical. Length 3.5 to 4μ, breadth 1.5 to 2μ.

NOSEMA SABAUNAE Lutz et Splendore 1908

[Fig. 99]

1908 *Nosema sabaunae* Lutz and Splendore 1908 : 314

Habitat: Undetermined Bombycidae (larva).
Locality: Brazil (São Paulo, Sabaúna).
Spore: Free or in cysts, in inconstant number. Regularly oval and refractive; with a vacuole at the rounded end. Length 6 to 7μ, breadth 2 to 2.5μ.

NOSEMA AURIFLAMMAE Lutz et Splendore 1908

[Fig. 100]

1908 *Nosema auriflammae* Lutz and Splendore 1908 : 314

Habitat: Imago of *Scea auriflamma* (Lepidoptera).
Locality: Brazil.

Spore: Found in a state of diffuse infiltration. Regularly oval with a vacuole at one end. Length 4 to 4.5μ, breadth 2 to 2.5μ.

NOSEMA MYSTACIS Lutz et Splendore 1908

[Fig. 101]

1908	*Nosema mystacis*	Lutz and Splendore	1908 : 314

Habitat: Two female *Ascaris mystax* from the intestine of a cat. The spores were found in the reproductive tubules which did not contain eggs or sperm.

Locality: Brazil.

Spore: Regularly oval and refractile: one vacuole at one end. Length 4 to 4.5μ, breadth 2 to 2.5μ.

NOSEMA DISTOMI Lutz et Splendore 1908

[Fig. 102]

1908	*Nosema distomi*	Lutz and Splendore	1908 : 314

Habitat: In the vitellaria of *Distomum linguatula* (?) from the intestine of *Bufo marinus*. The parasite was only found in those trematodes of toads collected from Guaringuetá.

Locality: Brazil.

Spore: Free or in cysts in variable number. Regularly oval with often a vacuole at one extremity. Length 2μ, breadth 0.8 to 1μ.

NOSEMA EPHEMERAE α Lutz et Splendore 1908

1908	*Nosema ephemerae* α	Lutz and Splendore	1908 : 314

Habitat: Intestine of nymph of *Ephemera* sp.

Locality: Brazil.

Spore: In diffused condition. Oval, rarely with vacuole. Length 3.5 to 4μ, breadth 2 to 2.5μ.

NOSEMA EPHEMERAE β Lutz et Splendore 1908

1908	*Nosema ephemerae* β	Lutz and Splendore	1908 : 314

Habitat: Same as the last form.

Locality: Brazil.

Spore: Either in diffused condition or in small cyst, containing 4, 8 or inconstant number of spores. Pyriform. Length 2μ, breadth 0.6μ.

Remarks: There are some points of resemblance between this form and *Stempellia mutabilis*.

NOSEMA CHIRONOMI Lutz et Splendore 1908

[Fig. 103]

1908	*Nosema chironomi*	Lutz and Splendore	1908 : 314

Habitat: In the larva of *Chironomus* sp.

Locality: Brazil.

Spore: Found in diffused state in the posterior part of the intestine. Pyriform with a vacuole at the broader end. Length 2 to 3μ, breadth 1.5 to 2μ.

NOSEMA EPHIALTIS Lutz et Splendore 1908

[Fig. 104]

1908	*Nosema ephialtis*	Lutz and Splendore	1908 : 315

Habitat: Imago of *Ephialtes angulosa* (Lepidoptera).

Locality: Brazil (Petropolis).

Spore: In diffused condition. Oval and ovocylindrical; without clear vacuole. Length 3.5 to 5.5μ, breadth about 2μ.

NOSEMA BALANTIDII Lutz et Splendore 1908

[Fig. 105]

1908	*Nosema balantidii*	Lutz and Splendore	1908 : 315

Habitat: In *Balantidium* sp. (Ciliata), parasitic in the croaca of *Bufo marinus.*

Locality: Brazil (Guaratinguetá and near São Paulo).

Spore: In diffused state or in a small cyst (with 4 or 8). Pyriform or oval with a pointed extremity. Length 2 to 5μ, breadth 1 to 3μ.

NOSEMA STEGOMYIAE Lutz et Splendore 1908

1908	*Nosema stegomyiae*	Lutz and Splendore	1908 : 315

Habitat: In the imago of *Stegomyia fasciata.* A polymorphic form.

Locality: Brazil.

Spore: Regularly or irregularly oval or pyriform. Found in the intestine in scattered condition or in cysts containing many spores. Length 3.5 to 7μ, breadth 2 to 2.5μ.

NOSEMA PULVIS Pérez 1905

[Fig. 106]

1905	*Nosema pulvis*	Pérez	1905 : 11-14, 18-22 1905a : 146-148 1905b : 150

Habitat: The muscle of *Carcinus maenas.* The muscle fibers showed atrophy. The blood of the infected host in which the schizogonic multiplication is undergoing actively, is white in color and less coagulable, while at the time of sporogony it becomes coagulable. In one case, the infection of the ovary was noted, and here the ova were reabsorbed by the follicle cells.

Locality: France (Arcachon).

Vegetative form: In cross-section of a muscle-bundle, the deeply stained spores fill the peripheral portion underneath the sarcolemma, while the central portion is not attacked by the microsporidian, exhibiting a state of diffused infiltration. In longitudinal section, fusiform groups of the spores are found between the muscular fibrillae. Each spore develops independently.

Spore: Ovoidal. The polar filament could not be observed. Length 1.25μ, breadth 1μ.

NOSEMA LONGIFILUM Hesse 1905

1905	*Nosema longifilum*	Hesse	1905a : 918-919

Habitat: Adipose tissue of *Otiorhynchus fuscipes* (Coleoptera).

Locality: France.

Vegetative form. Hesse: The microsporidian forms cysts which fill up the abdominal cavity. The host tissue reacts against the infection by developing a capsule of connective tissue around the cyst.

Spore: Spores of two kinds: 1) Oval and more frequently seen than the second. Some spores have a large vacuole at one end. The polar filament was extruded by treating the spores with Lugol's solution (instantaneously) or physiological salt solution (for one or two hours). Length 4 to 5μ, breadth 3μ, length of filament 85 to 90μ. 2) Elliptical and more voluminous than the first. They always seem to be empty. Length 6μ, breadth 4μ.

NOSEMA FRENZELINAE Léger et Duboscq 1909
[Figs. 107-113]

1909	*Nosema frenzelinae*	Léger and Duboscq	1909 : 773-734 1909a: 117-120

Habitat: Exclusively in the cytoplasm of *Frenzelina conformis*, a polycystid gregarine, parasitic in the gastric cecum and intestine of *Pachygrapsus marmoratus*. The microsporidian is found in the young, adult and encysted forms of the gregarine. It attacks, however, more abundantly the older forms and begins to sporulate when the host protozoon encysts. Ordinarily all the gregarines in one and the same crab are infected. The infected crab came from a limited locality. No harmful effect of the microsporidian upon the host gregarine was noticed, the encystment, formation of the gametes and the conjugation of the latter taking place in spite of a heavy infection. The crab tissues were free from the microsporidian infection.

Locality: France (Cavaliere).

Vegetative form: The microsporidian is easily recognized in stained preparations. Before the encystment of the host one finds the microsporidian in the protomerite. Under low magnifications the microsporidian appears as chromatic granules and resembles chromidia. This state of distribution naturally disappears in the encysted form. The schizonts are uninucleate, ovoidal and small (Fig. 108). They multiply actively by binary fission and form remarkably conspicuous chromatic strands in various parts of the host gregarines. At the end of the schizogony, each schizont develops into a single spore.

Spore: Extremely small (Fig. 112). The filament is coiled in the polar capsule. When extruded, the filament reaches 25μ in length (Fig. 113). The structure of the spore is similar to that of *Nosma bombycis* as observed by Léger and Hesse, that is, there are two nuclei in the sporoplasm and two which may be called as the nuclei of the shell-valves (Fig. 111). Dimensions: length (average) 2.8μ.

NOSEMA APIS Zander 1909

[Figs. 114 to 148; textfig. B, 4, 7]

1857	Fungus	Dönhoff and Leuckart	1857 : 66-67
			1857a : 199
			1857b : 210
1909	*Nosema apis*	Zander	1909 : 147-150, 164-166
1911	*Nosema apis*	Zander	1911 : 4-22
1911	*Nosema apis*	Fantham and Porter	1911 : 625-626
1912	*Nosema apis*	Fantham and Porter	1912 : 145-161
			1912a : 163-195
			1912b : 197-214
			1912c : 580-583
1912	*Nosema apis*	Maassen	1912 : 45-51
1913	*Nosema apis*	Fantham and Porter	1913 : 515-516
			1913a : 569-579
1913	*Nosema apis*	Darnell-Smith	1913 : 402-404
1914	*Nosema apis*	Imms	1914 : 62-70
1914	*Nosema apis*	Kramer	1914 : 481-514
1914	*Nosema apis*	White	1914 : 1-8
1916	*Nosema apis*	Beuhne	1916 : 629-632
1916	*Nosema apis*	Anderson and Rennie	1916 : 23-61
1916	*Nosema apis*	Ritchie	1916 : 160-161
1919	*Nosema apis*	White	1919 : 1-59
1920	*Nosema apis*	Fantham	1920 : 132
1921	*Nosema apis*	Kudo	1921 : 85-90
1921	*Nosema apis*	Koehler	1921 : 85-87
1922	*Nosema apis*	Morgenthaler	1922 : 53-60
1922	*Nosema apis*	Bullamore	1922 : 56-58

Habitat: In the midgut-wall and Malpighian tubules of adults of *Apis mellifica*. Fantham and Porter (1913) succeeded in producing experimental infections among the following insects: *Bombus terrestris*, *B. lapidarius*, *B. hortorum*, *B. venustus*, *B. latreillelus*, Mason bees, *Vespa* *germanica*, *Pieris brassicae*, *Callimorpha jacobeae*, *Abraxas grossulariata*, *Calliphora erythrocephala*, *Tipula oleracea* and *Melophagus ovinus*.

Locality: Cosmopolitan. England, U. S. A., Canada, Germany, Australia, Switzerland, Denmark, Tasmania, Natal.

The microsporidian frequently causes an epidemic disease among adult honey bees, known as Nosema disease, Nosema Seuche, Nosema-krankheit, Nosemasygdommen, etc. Fantham and Porter called the disease "Isle of Wight" disease. But it seems to be well established now that the so-called "Isle of Wight" disease in adult bees is due to an infection of the host bees by a microscopic mite, *Acarapis* (*Tarsonemus*) *woodi*. White (1914) found *Nosema apis* in the samples of bees received from 27 different States in the United States and in two samples of adult bees from Canada. This author (1919) mentions the effect of the climate upon the occurrence of the microsporidian in the United States as follows: "The infection was found in bees received from Florida and southern California, but in 15 samples received from Texas it was not found. The data thus far obtained indicate that less infection occurs in the southern portion of the United States than farther north. Whether it is found in the tropic or in the coldest climate in which bees are kept is not yet known."

The experimental infection upon the bees was carried by numerous investigators since Dönhoff first undertook it. It always shows a marked infection of the digestive tract only. Malden (1912, 1913) after studying the bacterial flora in the Nosema-infected bees, found that the number of bacteria in the infected bees were much greater than in norma ones, the proportion being 12:1. He, however, found no evidence of a direct etiological relation between these bacteria and the disease. White (1919) made some preliminary experiments with regard to the possibility of the presence of a filtrable virus in the disease, the results showing that no such a virus is present. As to the host organs attacked by the microsporidian, Zander stated that the midgut was the sole seat of infection. The infected gut distends to a volume twice as large as the normal gut, losing its reddish or brownish color and assuming a milky white coloration.

Fantham and Porter: *Nosema apis* is restricted almost entirely to the organs of the alimentary tract. The esophagus and the honey-sac are free from the attack, though ingested spores pass through the lumen of these parts and occasionally amoeboid planonts are found creeping over the lining of the crop and beginning to penetrate its cell. The chyle-stomach (mid-gut) is more easily infected than any other part of the gut. The amoebulae are found in all parts of the chyle stomach, but it is not common to find one part of the organ swarming with parasites while a short distance away the tissue is absolutely uninfected. The small intestine is also infected. The infection of this region is partly caused by the auto-infection of the spores from the chyle stomach. The hind-gut is rarely the seat of actual invasion by the Nosema, but its contents may be milky-white instead of yellowish on account of the Nosema-spores which have been shed into it. The colon and rectal regions of the gut are practically unattacked by the parasite. The Malpighian tubules seem to be free from invasion. Only on one occasion were meronts and a few spores found in one tubule only of one bee. Examination of the salivary glands and wax glands proved negative. The testis of the drone was free from infection, although their alimentary tracts were frequently infected. The ovary of the six queens examined showed negative except in one case where there were indications of Nosema, though three of the queens contained the spores in the gut. It is therefore questionable whether the eggs are infected or not, but as the queens are heavily infected, there is danger of the eggs becoming soiled with Nosema spores while or just after being laid. The planonts can pass through the intestinal wall and reach the hemocoelic fluid. The meronts and one or two spores (in one case) were also found in the fluid. Immature bee grubs of varying ages contain mostly meronts and a few spores in the cells of the mid-gut. The infection probably occurs by the contaminative method. The grubs from one particular infected hive appeared somewhat smaller than normal bee larvae. Muscular tissue appears free from the parasites, except in a few cases where the parasites develop near the muscles or against the sarcolemma of the muscles of the gut.

Maassen: The midgut is ordinarily the seat of infection. Malpighian tubules also become infected. Workers are in general infected, but in a few cases queens and drones were found parasitised, although the brood was always free from the infection. Experimentally workers, queens and drones become infected by *Nosema apis*. The microsporidian does not seem to multiply in the blood.

White: *Nosema apis* grows and multiplies for the most part in the epithelium of the stomach of the adult bee. Occasionally, but rarely, it is found within the epithelial cells of the Malpighian tubules. The parasite does not penetrate the basement membrane of these organs. Furthermore, the pharynx, esophagus, honey-sac, proventriculus, small intestine, large intestine (organs which possess a pronounced chitinized intima), blood, musculature or other organs of the host remain uninfected. Adult bees of all ages are susceptible to the infection. In heavily infected colonies the larvae and pupae apparently remain healthy. Larvae inoculated more or less directly by means of a pipette, showed upon daily examination (by sectioning) spores mixed with the food within the stomach from 1 to 3 days after inoculation, without any evidence that the parasite has increased in number or that it had invaded the tissues. The parasite is encountered most frequently in workers, although drones and queens are susceptible. In nature, it is not unusual to find from 10 to 20 per cent. of the workers of the diseased colones infected. Frequently a much higher percentage is met.

In no instance were the drones taken from the colonies in which the disease occurred in nature infected. In a few instances only were queens that were examined from such colonies found to be infected. As a result of artificial inoculation practically 100 per cent. of the workers of the experimental colony become infected. A very large percentage of drones also become infected. Queens in experimental colonies may or may not be found infected(8 infected out of 13 experimental colonies).

Vegetative form: Zander: The young amoebula that escapes from the spore membrane is a minute rounded body (Fig. 114) of about 2.8μ in diameter, which is exactly of the same structure with the planont of *Nosema bombycis* as worked out by Stempell. These planonts penetrate through the epithelial cell wall and become meronts which grow rapidly at the expense of the host cell substances. The meronts divide by binary fission repeatedly while the cytoplasm remains unseparated, resulting in formation of chain forms. That the growth and division of the meronts are quite rapid can be seen by the fact that after feeding normal bees with spore-containing honey, groups of spores are found in the epithelial cells of the midgut in 48 hours. When the nutrition becomes insufficient, the chain breaks into several daughter cells which become sporonts and develop ultimately into spores. The whole life cycle seems to be completed in 3 to 4 days under favorable conditions. Rapid and further infection of the healthy part of the intestine is made easy by the peculiar way with which the digestive fluid is secreted. The spherical epithelial cell filled with the spores is thrown out into the lumen of the gut and the lesions are quickly filled by a new cell. The spores isolated by the disintegration of the cell wall, will either pass out of the host gut with the fecal matter or will be the source of further infection of the newly formed epithelial cells in the host. The latter view is supported by the fact that the new epithelial cells which are formed become constantly infected.

Fantham and Porter: Each amoebula as it issues from the spore, shows two nuclei as refractile spots. The planont (Fig. 116, 117) moves by pseudopodia, one pseudopodium only being formed at one time and at one part of the body. The two nuclei may fuse together (karyogamy), after which the parasite creeps slowly between the gut-cells and ultimately penetrates them. Division of the uninucleate amoebula may occur while the parasite is free in the lumen of the gut. The cytoplasm may collect around each of the nuclei of an amoebula and then the division takes place. Each of these daughter forms may divide by binary fission or multiple division, each of which moves about over the surface of epithelial cells of the gut, and finally penetrates through between the cells or directly enters them and become intracellular form. Some of the planonts can also pass through between the cells of the gut and reach the hemocoel. The planonts, thus, become round or oval in form and are about 0.75 to 2.5μ in diameter.

Their movements are slow. The pseudopodia show occasionally an appearance of ectoplasm. Small vacuoles are sometimes present; the nucleus is small and consists of a small chromatin mass or karyosome suspended in a less dense substance, the nucleoplasm. Free planonts on the surface of the gut epithelium, or detached and so smeared, stain fairly well with Romanowsky stains. When they become intracellular, they stain only moderately, and can be distinguished with difficulty from the cell contents. The way of penetration of a host cell by a planont is hard to observe, though it has been seen in life on a few occasions. More than one planont can enter a single host cell. The planonts can be distinguished from the yeasts which may be present by their movements, by the affinity of their nucleus toward stain, and by chemical tests of which that for fungus cellulose is recommended. The planonts have no fungus cellulose. The planonts may reach the body cavity of the bee and remain there in a resting state for some time, that is to say, they lose their motility, become rounded or oval and lie quiescent. After an interval their activity returns, and from the hemocoel they retreat between the epithelial cells of the host gut, which they gradually penetrate. The planonts ultimately become passive, lose their pseudopodia and become meronts or schizonts.

The meront (Fig. 118) differs in structure from the planont in that the nucleus becomes gradually more chromatic and compact in nature. It increases in size, and then multiplies by merogony (schizogony). The multiplication is of three types: binary fission (Figs. 119-122), multiple binary fission and delayed multiple division. The large multinucleate meronts may be either intercellular or more commonly intracellular (Fig. 126). The tissue destructing power of the meront is very extensive, producing weakness and exhaustion and causing the death. Merogony is succeeded by two methods of sporogony. A single uninucleate meront becomes a sporoblast and then a spore. The cytoplasm is finely granular, the nucleus is distinct and at first single, though ultimately five nuclei are produced by divisions (Fig. 132). The meront when it transforms itself into a sporoblast undergoes the following changes. Its cytoplasm contracts to a slight extent and a thin spore membrane is secreted. In a large multinucleate meront, a gradual concentration of the cytoplasm takes place around each nucleus and the spore membrane is secreted. The nuclear division commences when the young spore shows two vacuoles and the sporoplasm assumes a girdle-like form. The nucleus becomes elongated and bowed; the chromatin substances concentrate into the ends, the nucleus becomes dumbbell in form and finally the ends become separated from each other. From one of the nuclei a bud is formed next, and the latter separates from the other half, passing toward the anterior vacuole (the nucleus of the polar capsule). The second nucleus from the original division divides into two small ones which are embedded in the sporoplasm. These nuclei control the growth of the shell, pass to the periphery, elongating itself and assuming a thread-like form. The remaining nucleus of the sporoplasm also divides into two, so that the sporoplasm contains two nuclei (Fig. 134). Fantham and Porter maintain that auto-infections of the bee with the spores occur, although limited to some extent.

Maassen: Auto-infection is unbelievable. The spores only undergo germination in a new host. The young stage, a large coccoid body, possesses mostly two nuclei and stains very easily and deeply. It multiplies by divisions, often forming a long chain. In the host epithelial cells, the amoeboid forms fuse with one another and form large multinucleated plasmodia. Plasmodia of 20μ long by 11μ broad are not rare. The plasmodium breaks up into sporonts. The sporont, maximum diameter 11μ, becomes divided into two sporoblasts and develops into two spores. The sporoblast has an ovoid shape, 10μ long by 5μ broad. The young spore possesses at first four nuclei and later two nuclei. If this is the case *Nosema apis* should be placed in the genus Perezia.

Spore: Zander: Oval; highly refractive (Fig. 115). The structure is identical with that of *Nosema bombycis* described by Stempell. Length 5μ breadth 2.8μ.

Fantham and Porter: Somewhat elongated (Fig. 137), usually oval; often pointed at one end. Highly refractile; a vacuole at each end. The sporoplasm is girdle-like in shape. The polar filament extends from the extreme anterior end of the spore to the opposite end. It passes as a straight rod backwards through the polar capsule and after reaching beyond the greater mass of the sporoplasm it becomes spirally coiled on itself in the vacuole (Textfig. B, 4). It is extruded by means of iodine water (Fig. 139) and dilute acetic acid. The mature spore contains two nuclei in the sporoplasm. Abnormal spores are often encountered. Length 4 to 6μ (occasionally 7μ), breadth 2 to 4μ, length of polar filament about 60μ.

Maassen: Size variable; average spores measure 6μ by 3μ. Some large ones are 14μ by 4μ. Mature spore contains a binucleate sporoplasm. Polar capsule was not observed. Extruded filament reaches 140μ in length. The filament is probably coiled directly inside the spore membrane.

White: More or less oval; of somewhat variable size. In India ink preparations, length 4.46μ, breadth 2.44μ. In iron hematoxylin, length 4.15μ, breadth 2.06μ.

Kudo: In fresh condition, the spore does not show any differentiation of its contents (Figs. 140-145). It is slightly less refractile than that of *N. bombycis*. The polar filament is invisible in life. When the spores are stained, the structure is similar to that of *Stempellia magna* (Figs. 146, 147). When the filament is extruded, it shows under certain circumstances, two regions: one with a regularly large undulation and the other with a uniformly small undulation, each having 10 to 15 turns (Fig. 148). This is interpreted as the polar filament of this microsporidian is coiled from 10 to 15 times along the polar capsule, inside of which and continuous to it, the filament is coiled back again toward the anterior end of the spore (Textfig. B, 7). Fresh spores 4.6 to 6.4μ long by 2.5 to 3.4μ broad and thick. Length of extruded filament is 230 to 280μ.

Morgenthaler: Length 5μ, breadth 3μ. Oval. Length of polar filaments extruded in hanging drop preparations reaches 400μ.

Kudo (1921, in April) showed that "the spore membrane . . . is proved to be composed of a substance similar to chitin in its chemical reaction." Koehler (1921, in July) also showed that "the spore membrane of *N. apis* is composed of chitin."

Methods of infection: Fantham and Porter: The method by which *N. apis* enters the bee appears to be purely contaminative either with the food or drink of the bee, or by the bees licking one another or removing drops of infective excrement from their own bodies, or absorbing spores during cleansing operations within the hive. Various insects also become infected and would become the source of infection in the bee.

White: At present there is no evidence that the infection of *N. apis* takes place otherwise than by way of the alimentary tract. This leads to the important tentative conclusion that the transmission of the disease is effected through either food or the water supply of the bees, or both. A sluggish body of water near an apiary and used by bees as a water supply, will be one of the probable sources of infection as the spores contained in the droppings of the infected bees falling into the water will be ready to infect new hosts. The robbing of diseased colonies is another source of infection. But the transmission of the disease through the medium of flowers is not to be feared. The hands and clothing of the apiarist, the tools used about an apiary, and the winds need not be feared as means by which the disease is spread. Hives which have housed infected colonies need not be disinfected and combs from such colonies are not a likely means for the transmission of the disease. Bees dead of the disease about the apiary are not likely to cause infection unless they serve to contaminate the water supply.

Imms: The bees contract the disease if fed with honey or syrup to which spores have been added, or with honey which has come from an infected hive. Experiments showed that healthy bees become infected (1) by contaminating their food with the excrement of diseased bees; (2) by placing bees which have died from the disease among them; (3) by confining them in cages which diseased bees had previously occupied; (4) by allowing them to feed on candy which had been previously utilised by diseased bees.

As to the ways by means of which the disease might be spread, water near the hives infected with bee excrement containing the spores seems to be a most important factor. Honey, pollen and wax, if contaminated with excrement containing Nosema spores, are fertile sources of infection. Infection from one hive or apiary to another is effected by the sale of diseased swarms, by the robbing of a diseased colony by healthy bees, and by swarms occupying old infected hives. Wet weather, especially when accompanied by cold, affords plenty of chances for bees to obtain moisture close to their hives, which becomes contaminated by the excrements discharged on the latter. There is evidence to indicate that partial immunity of stocks is found; such stocks might be difficult to diagnose, though they would at the same time act as sources of infection for susceptible colonies.

Symptoms and diagnosis of the diseased bees.

Zander: When the gut is heavily infected, and the intestine assumes the typical milky-white appearance, the disease can be diagnosed macroscopically. The spores are easily seen under low powers of magnification. It is difficult to definitely diagnose the infection when the gut does not show the typical change in coloration nor contain spores, in which case examinations of serial sections are the only means to determine the disease.

Darnell-Smith: Examination of the ground and grass around the infected hives shows a number of bees feebly crawling about, and exhibiting no desire to sting if molested. The abdomen of the bee is often distended. Distended abdomen, dysenteric discharge, falling from the alighting board, and a sort of paralysis and dislocation of the wings, are symptoms of the disease often observed.

Fantham and Porter: The question of symptoms is rendered very difficult because the bees vary enormously among themselves, so that there seems, at present, no one great outstanding symptom common to all. Infected bees crawl feebly about, evidently distressed. They do not try to fly away or to sting, when a finger is placed in front of them, simply climbing up on it. A distended abdomen is not always caused by the infection. Infected bees are incapable of defecating when on the wing and defecate when still. Infected bees will endeavor to perform their work as foragers, and while attempting to start a flight from the alighting board, it is common for them to fall from the board to the earth and die. Death occurs similarly among the infected foraging bees coming back from the field. While distended abdomen, dysenteric discharge, falling from alighting board and crawling are among the most common features, others are sometimes present, such as a sort of paralysis and dislocation of the wings. On some occasions an infected colony seems quite unable to produce normal wax, and the honey-comb may be rough, mingled with feces and undigested pollen, or sometimes it rapidly darkens, so that the comb would be considered to be several years old, whereas as a matter of fact it was newly made.

Some bees have survived attacks of disease and have become partially immune to the parasite, but when unfavorable conditions set in, the microsporidian overcomes the resistance of its host and the latter succumbs. Regarding the acquisition of partial immunity by bees, there is some hope of an immune race arising, exactly as has come about among silk-worms infected with *N. bombycis*. The authors state that one hive remained alive and flourishing from 1907 to 1912, although the hives on either side of it died out from Nosema-infection.

Imms: One of the earliest symptoms of the disease is the inability of most of the affected bees to fly more than a few yards without alighting. As the disease progresses the bees frequently can fly only a few feet from the hive, and then drop, and crawl aimlessly over the ground. They may be seen crawling up grass stems or up the supports of the hive. In heavily infected stocks great numbers of bees with distended abdomens may be seen crawling over the ground in front of the hives, frequently massed together in little clusters, while others remain on the alighting board. If the hives be opened, numbers of sluggish diseased individuals will often be met with inside, clustered together round or near the queen, who is usually the last to die. Diseased bees very frequently lose their power of flight altogether, and then crawl about with the extremity of the distended abdomen dragging along the ground; not frequently the wings are "out of joint," the hind wings protruding obliquely upwards and above the anterior pair. The distension of the abdomen appears to be due to the inability of the bee to fly. The hind intestine becomes loaded with pollen and other material, which is normally voided when the insect is on the wing. If, however, for any cause, it is unable to take its cleansing flight the hindgut remains loaded. In some cases, however, diseased bees show symptoms akin to those of dysentery. They discharge excrement over the combs and on the sides, floor and alighting board of the hive. Many bee-keepers have informed the author that this condition is only present after the winter confinement within the hive. A comb constructed by a diseased stock during the summer does not as a rule reveal any such dysenteric symptom. The only invariable feature is the death of large numbers of bees and frequently of the whole stock. The mortality is especially prevalent during wet and cold periods and during the winter season.

White: Nosema-disease presents only a few symptoms. The infected stomach shows a change in color. The brownish or dark reddish hue of the normal stomach is gradually lost as the disease advances and it becomes pale. The organ is often increased in size, the circular constrictions are less marked, and its transparency is diminished. In later stages of the disease, however, the stomach approaches the normal in size and the constrictions are again well marked. The organ is then white and opaque and the tissues are friable and easily crushed. When crushed the mass presents a milky appearance. The presence of the parasite is almost invariably recognized by its spore form and the presence of Nosema-infected bees in a colony is the only constant colony symptom of the disease. The bees, largely hybrids, grade Italians, Carniolans, Caucasians and a few common blacks, were susceptible to Nosema infection in all instances. Weakness, especially in the spring of the year, should arouse a suspicion that the disease is present. In order to make a definite diagnosis, however, an examination of the stomachs from adult field bees of the colony is necessary. Ten bees from a colony constitute a satisfactory sample as a rule. Ordinarily they are taken at the entrance with forceps and are killed by pinching the thorax. In removing the stomach for examination the bee is held by the thorax between the thumb and index finger of one hand and with a pair of forceps held in the other the tip of the abdomen is seized and pulled gently. By this method the organs of the alimentary tract forward to and including the stomach are easily drawn out. If the stomach upon removal appears swollen and lighter in color than a healthy one, Nosema infection may be suspected; if it is chalk-white and easily torn, infection is very probable; should the tissues of the organ when crushed be milky in appearance, infection is practically certain. Usually gross examination is sufficient for a definite diagnosis of the disease as encountered in nature. The diagnosis is completed when a microscope is used for the detection of the spores. To denote the degree of infection, slight, moderate, heavy, and very heavy should be used. Slight infection would indicate that not more than 10 per cent. of the bees are infected and that no noticeable loss is to be anticipated from the infection; moderate infection would indicate that from 10 to 35 per cent. are infected, that the colony will probably sustain losses from the disease, but that the chances are good for recovery; heavy infection would indicate that from 30 to 60 per cent. are infected, that the colony will most likely show weakness as a result of the disease, and that it may or may not die; and very heavy infection would indicate that more than 60 per cent. are infected and that the colony will probably die as a result of the disease.

Kudo: Heavily infected bees were characterized by their inactivity in the glass containers and by a peculiarly softened abdomen.

Preventive measures: Zander: Infected stocks should be burned. Healthy young bees may be isolated from the infected colony. The spores are infectious even after one year.

Fantham and Porter: Success in combating the disease seems to lie in preventive measures rather than in treatment. It is better by far to sacrifice the first stock showing signs of disease than to lose the whole apiary as a result of sparing the first set of weaklings. A supply of pure water in early spring, and generous treatment over winter food stores aid greatly in maintaining the vitality of the colonies, always a great factor in combating the disease. Diseased bees, whether they have died as a direct result of Nosema, or have been sulphured as a preventive measure, should be burnt, and the hives occupied by them destroyed or thoroughly disinfected.

Strong reagents such as creosote and pure lysol merely dissolve the outer layers of the spore, and do not seem to affect the contents within. To make an infected hive safe for a new stock, use a painter's lamp over all the woodwork of the hive, and burning of the top soil around the hive, followed by liming of the soil for some distance around is necessary. Great precaution must be taken when the bees are imported from another country where the disease occurs frequently, as Nosema-carriers may be the center of a new and heavy infection under favorable conditions.

Beuhne: Not to locate the hives in shady situation; to keep the ground around the hives bare and clean; to keep the hives dry during the winter; to requeen all the colonies which, from no visible cause, lag behind the average, and are therefore possibly disease-carriers; to use for re-queening only queens from stocks which, by their yields of honey due to longevity of the workers, have proved their resistance to the disease.

White: Nosema spores suspended in water are destroyed by heating for 10 minutes at about 58° C. Suspended in honey, they are destroyed by heating at about 59° C. The spores, drying at room and outdoor temperature, remained virulent for about 2 months, at incubator temperature about 3 weeks, and in a refrigerator, about 7 months and a half. *Nosema apis* was destroyed in the presence of fermentative processes in a 20 per cent. honey solution in 3 days at incubator temperature and in 9 days at outdoor temperature. In a 10 per cent. sugar solution it was destroyed in from 7 to 11 days at the room temperature. The parasite resisted putrefactive processes for 5 days at incubator temperature, for 2 weeks at room temperature, and for more than 3 weeks at outdoor temperature. The parasite when dry was destroyed in from 15 to 32 hours by direct exposure to the sun's rays. The spores suspended in water were destroyed by exposure to the sun's rays in from 37 to 51 hours. When suspended in honey and exposed to the sun's rays frequently, the spores would be destroyed due to the temperature of the honey. The spores remained virulent in honey from 2 to 4 months at room temperature. The spores in the bodies of dead bees ceased to be virulent in one week at incubator temperature, in 4 weeks at room temperature, in 6 weeks at outdoor temperature and in 4 months in a refrigerator. The spores contained in the bodies of dead bees lying on the soil ceased to be virulent in from 44 to 71 days. The spores are easily destroyed by carbolic acid, a one per cent. aqueous solution destroying them in less than 10 minutes. The virulence was based upon the results of feeding normal bees upon the experimental material.

Imms: The most satisfactory measures so far discovered are preventive rather than curative. Healthy stocks should be removed from the neighborhood of diseased hives. The water supply should be rigidly attended to; clean water changed daily should be readily accessible and protected from contamination. The usual drinking places should be removed if possible. All dead bees should be burnt and diseased colonies destroyed. The ground around the hives should be dug over and treated with quick lime. Infected hives and the parts associated with them should be charred with a painter's lamp. In the place of charring a very thorough application of formalin or carbolic acid may be used, and the hives afterwards properly aired in strong sunlight.

NOSEMA SCHNEIDERI Léger et Hesse 1910

1910	*Nosema schneideri*	Léger and Hesse	1910 : 411-412

Habitat: Epithelium of intestine of nymph of *Ephemera vulgata*. The parasite does not cause any noticeable hypertrophy of the host cells.

Locality: France.

Vegetative form: The schizonts are spherical and 2μ in diameter. They multiply by binary fission and become monosporous sporonts. When the spores are fully formed, they reach the lumen of the intestine.

Spore: Ovoidal. A small chromatic calotte is present at one end from which the filament becomes extruded. Length 4μ, breadth 2μ, length of polar filament 90μ.

NOSEMA BRANCHIALE Nemeczek 1911

[Fig. 149, 762]

1911	*Nosema branchiale*	Nemeczek	1911 : 163-164

Habitat: Branchial lamella of *Gadus aeglefinis*. Three infected fishes were examined.

Locality: Austria (fishmarket in Vienna; April).

Vegetative form: Spherical "cyst", 0.2 to 0.5 mm. in diameter is embedded in the lamella; in one case, it was oval and measured 1 mm. long. In sections, the ectoplasm is stained very deeply, and does not show any fibrillar structure as that of *Glugea anomala*.

Spore: Oval with a very distinct vacuole at one end. The sporoplasm is finely granular. The filament is extruded under the influence of mechanical pressure. The polar capsule and filament were stained with gentian violet, but not with Löffler's method (which is not the case with *Nosema bombycis*, after Kudo). Length of fresh spores 6.3μ, breadth 3.5μ, polar filament 90μ long.

Remarks: Nemeczek's observations upon the vegetative form are inadequate to assign this form definitely to this genus. Since he compares the "cyst" with that of *Glugea anomala*, he may have had a species of Glugea although he designated it as a Nosema. It is listed here provisionally.

NOSEMA LEGERI Dollfus 1912

[Fig. 150]

1897	Glugéidée	Giard	1897 : 957
1897	*Plistophora* sp.	Léger	1897 : 957-958
1912	*Nosema legeri*	Dollfus	1912 : 125-129

Habitat: Parenchyma of *Brachycoelium* sp. (Trematoda), parasitic in *Donax trunculus, Tellina fabula, T. teunis, T. solidula* (*balyica*) (after Giard and Léger) and various tissues of meta-cercariae of *Gymnophallus somateriae strigatus* (Trematoda), parasitic in *Donax vittalus* (after Dollfus).

Léger: More than half of the trematodes were infected by the microsporidian. Infected individuals were chalky-white in coloration and were larger and more rounded than those not infected.

Dollfus: When a metacercaria taken from Donax is infected, almost all the others from that Donax were found to be infected by the microsporidian. The microsporidian does not infect the Donax so that it is beneficial for this mollusc in that the microsporidian has a fatal effect upon the trematode. Lightly infected trematodes are transparent and a little colored. Normal translucent trematodes are active and show clearly the internal organs, while the opaque white metacercariae are immotile, uniformly granulated and usually dead. Moderately infected ones show feeble movements and their organs more or less visible. When heavily infected, the metacercariae are spherical in form and dead in which condition they appear as sacs loaded with spores.

Locality. France.

Vegetative form. Léger: Young stages were difficult to observe. Spores were usually in spherical masses which measured 15 to 20μ in diameter. These groups were surrounded by an extremely fine membrane and contained spores variable in number.

Dollfus: Meronts either in the resting state or undergoing simple or multiple schizogony were observed. One point which the French author established is that a sporont gives rise to a sporoblast which in turn develops into a single spore. The spores are diffused in the tissues of the host trematode.

Spore. Léger: Small; ovoidal; one end more rounded than the other. At the rounded end, there is usually seen a clear rounded vacuole. Length 5μ, breadth 2.5μ.

Dollfus: Ovoidal or rounded (Fig. 150); very refractile; with a clear vacuole at the rounded extremity. The cytoplasm is uniformly stained. Polar capsule, filament and valve-nuclei were not noted. Length 5μ, breadth 2.5μ.

NOSEMA PULICIS Nöller 1912

[Figs. 151-153]

1912	*Nosema pulicis*	Nöller	1912 : 525 1912a : 396
1914	*Nosema pulicis*	Nöller	1914 : 310

Habitat. In *Ctenocephalus canis.*

Nöller noticed that about 6 per cent of the host insect studied by him in Berlin were infected by the microsporidian, that the seats of the infection was the epithelium of the ventriculus, Malpighian tubules, fat body and salivary glands, that in female hosts the ovaries were also infected so that germinative infection takes place and that "it causes the death of the host flea in the case of heavy infection" (1912) or "in spite of an abundant presence of the parasites in the epithelium of the midgut, the host fleas are unaffected and live over one month even if very heavily infected." (1912a).

Locality. Germany (Berlin).

Spore: Oval. Dimensions: 2.5 to 5μ long by 1.5 to 2μ broad. The length of extruded polar filament is 65 to 85μ.

Remarks: Compare with *N. ctenocephali.*

NOSEMA GLOSSIPHONIAE Schröder 1914

[Figs. 154-157]

1914	*Nosema glossiphoniae*	Schröder	1914 : 323-324

Habitat: The muscle cells of *Glossiphonia complanata* (Hirudinea). One infected animal was observed. The posterior portion especially on one side of the body, was whitish in color.

Locality: Germany (Heidelberg March).

Vegetative form: Smears and sections did not show any stages of multiplication.

Spore: Ellipsoidal, but form and size are variable (Figs. 154-157). The polar filament was extruded only in one case. The sporoplasm, a broad ring in form, contained always two nuclei. A portion of the filament could often be noticed at the anterior end of the spore. Dimensions: length 4μ (a few 6μ), breadth 2.5μ (a few 3μ), length of the filament extruded was one and half time that of the spore. This is without doubt the incompletely extruded filament.

NOSEMA BOMBI Fantham et Porter 1914

[Figs. 158-177]

1914	*Nosema bombi*	Fantham and Porter	1914 : 623-638

Habitat: Alimentary canal and Malpighian tubules of *Bombus agrorum, B. hostrum, B. latreillelus, B. lapidarius, B. sylvarum, B. terrestris.*

It may also pass naturally to *Apis mellifica* and to *A. florea*. The microsporidian is pathogenic to the above hosts.

In certain districts every *Bombus agrorum* was found to be infected, *B. terrestris* was also fairly often infected, while only small number of the other species of the host were found to be parasitised. Infected insects frequently lose their power of flight, and crawl in a blundering manner over the ground. They are easily irritated, and sting on far less provocation than usual. The parasite is found mainly in the Malpighian tubules, and to a less extent in the alimentary canal. At earlier infection, the chyle stomach alone may be parasitised, but the infection rapidly spreads to the Malpighian tubules (in 48 hours after the artificial infection *per os*, the Malpighian tubules become infected). The small intestine becomes infected after or simultaneously with the excretory tubules, but the infection of the gut is never so severe as it is in the case of a hive bee infected with *Nosema apis*. The fat body of infected insects is always much reduced in bulk compared with that of normal ones. The hemocoelic fluid appears more abundant in diseased than in healthy bees. Infections of the Malpighian tubules and the intestine seem to play a subsidiary part in bringing about the death of the host. The ovaries of an infected queen are often smaller than those of a normal one; but the genital organs of both male and female bees do not seem to be affected by the microsporidian and there is no evidence of hereditary infection. This microsporidian possesses in the opinion of the authors an important economic significance, since the Bombi are essential for the fertilization of certain plants of agricultural and chemical importance, the red clover being the best known. The death of the Bombi due to the effect of an infection of *Nosema bombi* has resulted in less red clover seed in certain districts. The possibility of the contraction of microsporidiosis of humble bees by hive bees is also not without significance.

Vegetative form: When the spores are swallowed by a bee, they remain relatively unchanged until they reach the chyle stomach. Then the spore membrane becomes softened, an anchoring filament is extruded and fixes the spore to the gut-wall, and the sporoplasm within creeps out as a small motile body termed amoebula or planont (Fig. 158). Each planont is a very small organism, about 2.2μ in diameter, capable of active movements by means of a pseudopodium. It is first binucleate; by division, two uninucleated planonts are formed. The cytoplasm is finely granular, while the nucleus may be homogeneous or may show a karyosome. The motile period is short, and the planonts penetrate a cell of the lining of the gut or work their way to the Malpighian tubules of the host, becoming the meronts.

The meronts (Fig. 159) vary considerably in size and shape. Usually oval or rounded, they become elongated, heart-shaped or irregular, when they divide. The nucleus may be vesicular with a karyosome, or have its chromatin in the form of relatively evenly distributed grains. Extranuclear chromatin is rarely present. The cytoplasm of the large meronts shows fine alveolar structure. A division of the meront is initiated by a simple division of the nucleus, of a promitotic type. There is concentration of the chromatin at opposite poles of the nucleus, and then gradually the ends diverge, a strand frequently connecting the two nuclei for some time (Figs. 161, 163). At other times the strand is very short, or may appear to be suppressed. The daughter meronts vary greatly in appearance, being oval, elongated oval, etc. As the rate of division differs, three or four nuclei are produced at one time. Cytoplasmic constriction does not necessarily follow nuclear division so that multinucleated forms (Figs. 167-171) are formed especially when the space in which they multiply is limited. The number of meronts in the gut is not so large as it is in the Malpighian tubules. When the host is becoming exhausted, the meronts lose their power of division and each gives rise to a single spore (Figs. 172-177). The nucleus divides into two, one of which divides again, while the other gives rise by divisions to three nuclei (Fig. 176). These changes are similar to those in *N. apis* as described by the same authors. At the same time, two vacuoles are formed, one at each end of the spore; and the sporoplasm assumes its typical girdle-like shape (Fig. 176). Within a vacuole, the polar filament is produced; it passes backwards and coils up in the posterior vacuole (Fig. 177).

Spore: Length 5.2μ (often 6.5 to 7μ), breadth 3.7μ. The dimensions are less dissimilar than in *N. apis*. The younger the host, the larger the spore. That there is but one parasite concerned is proved by cross-infection experiments. Old Bombi fed on the strain of large spores become infected, but the spores are often smaller. When such strains of small spores are fed to young Bombi or to hive bees, the larger form of spores reappears and predominates. The polar filament is extruded through a foramen in the spore membrane under the influence of the digestive fluid of a new host. Artificially, ejection of the filament can be induced by the action of nitric acid, acetic acid, iodine solution or glycerine.

NOSEMA CTENOCEPHALI Kudo 1924
[Figs. 178-182]

1916	*Nosema pulicis*	Korke	1916 : 725-730

Habitat: Digestive tract of *Ctenocephalus canis* (*C. felis*).

The flea was taken from a half-bred spaniel. About 17 per cent. were infected. Korke fed the flea on wild rats. Every other larva examined after about three weeks, was infected. Early infection occurred in the midgut. Heavily infected larvae were dark and mottled in appearance, by which they were at once distinguished from the healthy. Infection takes place throughout the digestive tract, i.e., from esophagus to rectum. Massive infection was quite compatible with movements and activity of the larva.

Locality: India (Kasauli).

Vegetative form: The life-cycle of the microsporidian is much similar to that of *N. bombycis* as worked out by Stempell (1909). The amoebulae are not more than 0.75μ in diameter and it is difficult to detect their presence, form and movements. Two nuclei of the amoebula (Fig. 182) fuse into one, thus forming a uninucleate planont, 0.1 to 0.75μ. When active, the movements of the planont are fairly rapid, though actual protrusion of a pseudopodium is not easily recognized. When stained, a blunt pseudopodial protrusion appears to arise from one pole of the cell and in one direction only. After entering host cells, the planonts become meronts which multiply either by binary fission or by multiple division. Each meront becomes a sporont. The sporont shows the following structures when stained: two parietal nuclei, a polar capsule with a filament, and a sporoplasm ("spore") which is uninucleate, but without a vacuole.

Spore: Oval; refractile in fresh state [Fig. 178). One or two vacuoles may be made out at the extremities. In some cases, one finds an actively mobile (?) refractile spot. The polar filament is extruded under the influence of weak acetic acid or iodine solution (Figs. 181, 182). The sporoplasm contains a single nucleus (Fig. 180). As to the function of the polar filament Korke thinks that besides fixing the spore to the gut-epithelium it serves to conduct the amoebula to a distant part of the tissue and thus to ensure the advance of the parasite into fresh areas. Length up to 1.5μ; the extruded filament is 25μ long.

Remarks: Korke apparently did not know Nöller's observations upon a microsporidian parasitic in the dog flea, to which Nöller gave the name, *Nosema pulicis* and named the present species *Nosema pulicis* which, of course, cannot hold for a new species. Whether or not this Indian form is identical with the Berlin form cannot be determined, since Nöller's description is brief. The dimensions of the spores are exceedingly different in these two forms; therefore they are here recorded separately. I have given the Indian form the name, *Nosema ctenocephali*, in place of Korke's name. Korke gave *Ctenocephalus felis* for the host flea which he called the dog-flea. It is possible that this was *Ctenocephalus canis* instead.

NOSEMA sp. Ishiwata 1917

[Figs. 183-186]

1917	*Nosema* sp.	Ishiwata	1917 : 136-137

Habitat: Larvae of *Attacus cynthia*. The infection was fairly common.
Locality: Nippon (Tokio).

Spore: Ovoid, tapering toward both ends (Fig. 183); less refractive than *Nosema bombycis*. The polar filament is shorter and thicker than that of *N. bombycis*, and has a round knob at its distal end which is seen more frequently than in the latter species (Figs. 184, 185). The polar filament is coiled longitudinally in the capsule, often exhibiting concentric lines while in the spore (Figs. 185, 186). The filament is sometimes under mechanical pressure extruded from the side of the spore and takes the form of a ring, which is probably due to the adhesive round end. Length 3 to 3.5μ, breadth 2μ, the filament is not measured.

Remarks: The author does not give any characteristics of the vegetative form which may justify placing the species in the present genus. It is therefore placed here provisionally.

NOSEMA CULICIS Bresslau 1919
[Fig. 187]

1919	*Nosema culicis*	Bresslau and Buschkiel	1919 : 326-327

Habitat: In a larva of *Culex pipiens*. The microsporidian spores were noticed in a smear of the contents of a larva.

Locality: Germany (Frankfurt a. M.?).

Vegetative form: Undescribed.

Spore: Elongated ovoid; one end is truncated, the other end is broadly rounded (Fig. 187). Length 4.5 to 5.5μ, breadth 1.8 to 2.4μ. The polar filament is not mentioned.

Remarks: Bresslau does not describe the vegetative form. It is listed as a Nosema provisionally.

NOSEMA sp. Nöller 1920

1920	*Nosema* sp.	Nöller	1920 : 187

Habitat: Larvae of *Aëdes nemorosus* and *A. cantans*.

Locality: Germany (Hamburg).

Vegetative form: Undescribed.

Spore: Somewhat broader and shorter than that of *Nosema culicis*.

Remarks: This is a doubtful form with no clear generic designation. It is listed here provisionally.

NOSEMA sp. Martini 1920

1920	Nosema	Martini	1920 : 34

Habitat: *Aëdes* sp.

Locality: Germany (in the vicinity of Hamburg).

Remarks: Another uncertain form with ambiguous generic designation. It is listed here provisionally.

NOSEMA BAETIS Kudo 1921

[Figs. 188-205, 772-774]

1921	*Nosema baetis*	Kudo	1921a : 171-176

Habitat: Fat-body of nymphs of *Baetis* sp. (?). Out of 42 nymphs, eight were infected by the microsporidian. The thorax of the infected nymph was strikingly whitish opaque, and was more or less distended. The infected nymphs were very inactive and easily caught by means of a pipette, since the muscular tissue in most cases was pushed aside due to the immense growth of the infected adipose tissue, and showed atrophy or poor development even though the effect was indirect. The microsporidian could not be transmitted to young larvae of *Culex pipiens*, when the latter were kept in a water emulsion of infected Baetis nymphs.

Locality: The United States (Urbana, Illinois).

Vegetative form: The youngest schizont found in the host fat body, is a small rounded body about 3μ in diameter, and contains a comparatively large nucleus. In general appearance, it resembles the corresponding stage of *N. bombycis*. Schizogony is binary fission. The nuclear division seems to be amitotic. The schizogonic divisions (Figs. 188-194) take place repeatedly until the host cells are filled with the schizonts which now become the sporonts (Fig. 195). The sporont becomes elongated and the cytoplasm condenses into a girdle form at the middle of the spore. The polar capsule seems to become differentiated nearly in the center of the spore (Fig. 203), and is best seen in the spores stained after Fontana. A nucleus for the spore membrane was not noticed. Young spores are slightly larger than the mature ones and these two types show different affinities toward stains.

Spore: The spore is elongated oval (Figs. 200-205), with often dissimilar extremities. Fairly refractile. The mature spores are 3 to 4μ long by 1.5 to 2.5μ broad. Length of extruded filaments 94 to 135μ. The structure of the spore seems to be similar to that of *Nosema bombycis* as described by Kudo.

NOSEMA CYCLOPIS Kudo 1921

[Figs. 206-210]

1921	*Nosema cyclopis*	Kudo	1921b : 137-138, 140

Habitat: Fat bodies and reproductive organs (?) of *Cyclops fuscus*. Two, one male and the other female, out of twenty-two host individuals were found infected by the microsporidian. The infected animals were strikingly whitish opaque in color compared with the normal ones. The host did not suffer any decrease in activity. This is probably due to the fact that the parasites do not attack the muscle cells. But there seemed to be little doubt as to the fatal outcome of the infection.

Locality: The United States (New York); August.

Vegetative form: The youngest schizonts are rounded bodies, each with a single deeply stained chromatic mass. The body increases in size as the nucleus multiplies repeatedly, forming spherical, oblong or elongated bodies with 2, 3, 4, 5 or 6 nuclei. Each sporont develops into a single spore.

Spore: Pyriform; anterior end rounded at tip; posterior end broadly rounded (Figs. 206,207). The spore membrane is very thin. Less refractive. In cross-section, the spore is circular (Fig. 208). The broadest portion of the spore is located near the posterior end. In the fresh state, there is always seen an oval clear space at the posterior end; the rest of the spore is filled with finely granulated cytoplasm. When stained, the sporoplasm and the capsule with its coiled filament become plainly visible (Figs. 209, 210). Fresh spores 4.2 to 4.7μ long by 2.7 to 3μ broad. Length of extruded filaments 75 to 100μ.

NOSEMA INFIRMUM Kudo 1921
[Figs. 211-218]

1921	*Nosema infirmum*	Kudo	1921b : 138-140

Habitat: Fat body, reproductive organs and muscles of *Cyclops albidus*. This microsporidian was observed in 1 out of 12 crustaceans from one locality and in 21 out of 153 collected from another locality. *Cyclops fuscus* found in the infected locality proved to be free from the infection of the present species. The infected animals were as strikingly whitish opaque in color as in the case of Cyclops infected by *N. cyclopis*. However, they showed a marked decrease in activity compared with normal ones; while apparently normal individuals were difficult to capture with a pipette, the parasitised individuals were easily caught by the same means.

The effect of the parasite upon the host body seemed to be fatal. In the collection from the second locality, 15 dead host animals were found completely filled with spores and extremely whitish opaque in appearance, a condition which made such animals conspicuously visible against the brownish bottom soil of the aquarium in which they were kept.

Locality: The United States (New York); August and September.

Vegetative form: Very similar to the last mentioned species.

Spore: Pyriform; the anterior end is more or less rounded at the tip, while the posterior end is either more pointed or rounded than the anterior (Figs. 211-213). The shape of the spore is, however, distinctly different from the last species. The spore membrane is thin, though slightly thicker than in *N. cyclopis*. In cross-section, the spore is circular (Fig. 214). The broadest part is in the middle of the axis of the spore. Fresh spores show an oval or irregularly triangular space at or near the posterior extremity of the spore. The sporoplasm (Figs. 215-218) is stained in the posterior half of the spore. The filament is more easily recognized than the last mentioned species. Fresh spores measure 5.6 to 6.4μ long by 3μ thick. The filament is 90 to 115μ long.

NOSEMA ANOPHELIS Kudo 1924
[Figs. 728-730]

1924	***Nosema anophelis***	**Kudo**	**1924a (in press)**

Habitat: In a larva of *Anopheles* sp.; in epithelial cells of the gastric pouch of a larva of *A. quadrimaculatus* and in epithelial cells of the anterior portion of the midgut and in fat body close to the midgut of an engorged adult *A. quadrimaculatus.*

The first host larva was also slightly infected by *Thelohania legeri.* In the second host larva, a number of the epithelial cells of the gastric pouches were infected by various stages of the microsporidian. In the third host, the ova which were well developed, were apparently free from the infection.

Locality: The United States (Leesburg, Georgia; August, September).

Vegetative form: The young schizonts found in the host epithelial cells are about 1.5μ in diameter and each possesses a relatively large vesicular nucleus. Schizogony is binary fission (Fig. 728), and seems to take place repeatedly. Spore formation begins before the host cell becomes completely filled with the schizonts. Each schizont transforms itself into a sporont and finally into a spore.

Spore: Oblong; one end slightly narrower than the other. Frequently the sides are asymmetrical (Fig. 729). Moderately refractive. In many spores, a large vacuole is to be seen at one end. The membrane is thin and weak and a slight pressure of the immersion objective upon the cover glass causes filament extrusion. Fresh spores measure 4.7 to 5.8μ long by 2.3 to 3.2μ thick; the filament 50 to 60μ long. Fixed and stained spores: from the first host, 4 to 5.2μ long by 2.5μ broad; from the second host, 4 to 5.5μ long by 2.2 to 2.5μ broad; from the third host 4 to 5μ long by 2 to 2.5μ broad.

Genus GLUGEA Thélohan emend. Weissenberg

The characters of the genus are described on page 66.

Type species: *G. anomala* (Moniez) Gurley 1893.

GLUGEA ANOMALA (Moniez 1887) Gurley 1893
[Figs. 219-264, 761]

1838		Gluge	1838 : 772
1887	*Nosema anomala*	Moniez	1887a : 1312-1313
1892	*Glugea microspora*	Thélohan	1892 : 174
1893	*Glugea anomala*	Gurley	1893 : 193
1895	*Glugea microspora*	Thélohan	1895 : 133, 139, 356,
1899	*Nosema anomalum*	Labbé	1899 : 105
1904	*Glugea anomala*	Woodcock	1904 : 58
1904	*Nosema anomalum*	Stempell	1904 : 1-42

1911	*Glugea anomala*	Awerinzew and Fermor	1911 : 1-6
1911	*Glugea anomala*	Weissenberg	1911b : 346-351
1913	*Glugea anomala*	Weissenberg	1913 : 81-163
1914	*Glugea anomala*	Weissenberg	1914 : 380-389
1920	*Glugea anomala*	Debaisieux	1920 : 217-243
1921	*Glugea anomala*	Weissenberg	1921 : 400-421

Habitat: *Gasterosteus aculeatus*, *G. pungitus* and *Gobius minutus*.

Thélohan (1895): In the subcutaneous connective tissue and cornea of *Gasterosteus aculeatus* and *G. pungitus* and once in the ovary. Connective tissue of *Gobius minutus* (Henneguy).

Stempell (1904): In the subcutaneous connective tissue, ovary, peritonium and alimentary canal of *G. aculeatus* and in the integument of *Gobius minutus*.

Weissenberg (1913): In both fresh water and marine forms of *G. aculeatus*. The cysts reach 3 to 4 mm. in diameter. If they occur in the integument, the body becomes greatly deformed. If present in the body cavity, they often fill the latter completely, the ovary and the intestine becoming completely pressed out of their normal situation. Unlike the cysts of *Nosema lophii*, those of *G. anomala* are not connected with any definite organ systems; they occur in the connective tissue of the integument, in the substantia propria of the cornea, in the gut-wall, in the liver or in the connective tissue of the ovary and testes. In the skin, cysts may occur in the head, on the sides or on the ventral surface, or on the fins and wall of the branchial chamber.

Debaisieux (1920): in paired or unpaired fins, wall of the branchial cavity, cornea, and other part on the surface of the body, but not in internal organs.

Weissenberg (1921) succeeded in producing experimental infections by *Glugea anomala* in very young fish and found that the parasite enters the host cell, undergoes growth and multiplication as a result of which the host cell becomes extremely hypertrophied and forms a so-called "Glugea-cyst" and that the so-called "vegetative nuclei" of the parasite are none other than the host cell nuclei. Debaisieux also came to a similar conclusion as to the real meaning of the vegetative nuclei.

Locality: France, England, Russia, Germany, Belgium.

Vegetative form: Stempell (1904): The youngest stage is a small, multinucleated protoplasmic mass with a thin membrane. The cyst later becomes surrounded by a thick membrane and also by a capsule of the connective tissue of the host. As the parasite grows, the vegetative nuclei also grow often becoming elongate. From the protoplasm are differentiated uninucleate primary sporonts each with a thin membrane, the nucleus being formed from the vegetative nuclei. These sporonts lie in clear spaces which later join together, and form a large central space. The sporonts either develop directly into spores or break up by successive divisions into various and numerous uninucleated secondary sporonts. The spore differentiates a membrane and two vacuoles. The nucleus after giving off chromatin substances divides into four nuclei of which two are the nuclei of the polar capsule and the other two, of the sporoplasm (Fig. 260). In the polar capsule is coiled a filament 150μ long. After the spore is completely formed the vegetative nuclei break up into small granules. In older cysts, there occurs a dissolution of the cyst membrane, in which case small secondary protoplasmic bodies are formed in the protoplasm. The fine chromatic granules reconstruct the nucleus in each of these bodies. These secondary bodies migrate into the host tissue, and develop into sporonts and spores as the primary sporonts. Stempell tried to fill up the gaps in the life cycle of the microsporidian by comparing it with that of *Thelohania mülleri* as follows: When a mature spore enters the digestive tract of a new host, the filament is extruded. Two uninucleated sporoplasms copulate and the copula, after leaving the spore, becomes a multinucleated cyst by growth upon entering the intestinal wall.

Awerinzew and Fermor: When the parasite is of enormous size, the region with mature spores become divided into chambers with a very fine protoplasmic wall. These vacuoles are of independent nature until the cyst breaks up (Fig. 226). The wall becomes finer as the parasite grows. The primary sporonts are formed in the following manner (Figs. 227-231): at the periphery of large cysts, there are relatively large nuclei containing numerous chromatin granules, embedded in vacuole-free protoplasmic layer. They grow, become elongated in one direction (radially) and assume a sausage form, the part near the end directing toward the center of the cyst containing less chromatin than the other (Fig. 227). The chromatic granules break up in separate groups and from one end of the body, they become transformed into nuclei of the vesicular type. These nuclei divide again. The vegetative nuclei are independent and finally degenerate or give rise to the nuclei of the meronts. The cytoplasm of the meronts stains differently. The sausage form breaks up into as many small bodies as the nuclei (Fig. 231), each of which gives rise to a sporont and later to a spore.

Weissenberg (1913): The starting point in the cyst is the primary nucleus around which the cytoplasm becomes gradually concentrated and the nucleus together with its cytoplasm forms the primary body (Textfig. E1). From this an octonucleated body is formed by nuclear divisions and elongation of the body (Textfig. E4, 5, Fig. 242). This body breaks up into eight uninucleate bodies in the vacuole which becomes differentiated around them (Fig. 243). These bodies (Fig. 248) are called "vacuole-cells" by Weissenberg. They divide (Figs. 249, 250) into two simultaneously so that after the completion of the divisions there are 16 spherical sporoblasts (Textfig. E 9) in the vacuole. Each sporoblast becomes elongated, its nucleus assumes an eccentric position and at the opposite pole there appears a thickening of the filament. The nucleus returns towards the center of the body and at the same time there appears a large vacuole at the rounded extremity. Thus from 16 sporoblasts are formed 16 spores.

Debaisieux (1920): The tumor is lodged in the epidermis between bundles of fibrous connective tissue cells rich in connective substances. Lacunae usually filled with blood border the cyst wall. There exists a stratum of modified connective tissue in concentric layers. Around the tumor there is to be found a continuous membrane of 5 to 10μ in thickness. It is very selective to stains and appears more or less hyaline after staining. It seems to be probable that the tumors are formed by parasitised and hypertrophied host cells, rather than parasites. In a tumor three zones are to be distinguished: a) Immediately under the membrane, a continuous protoplasmic layer of variable thickness is seen. The cytoplasm is alveolated and contains very minute granules surrounded by clear spaces. b) In the second zone, there are numerous nuclei of two kinds. The large ones are either spherical or irregularly elongated as if they were on way of degeneration. Among these large nuclei, smaller ones are found. They are surrounded by small islands of cytoplasm and some are united in plasmodia which are highly elongated or spherical in form. At the inner part of this zone, great vacuoles are found. The vacuoles contain uninucleated individuals formed by the fragmentation of the plasmodia and stages of sporulation. c) The central portion of the tumor is filled with mature spores. Membranes which surround groups of spores disappear in the center. This region occupies the largest portion of the tumor. Its diameter is ten to twenty times the thickness of the peripheral zone.

Young schizonts are uninucleated; the nuclei are almost always undergoing division. Two granules are noticed at the poles. The chromatin granules gather at the equatorial plane. In a phase which may be called the anaphase, each pole possesses two chromatin grains which are connected by two chromatic filaments. Nuclear divisions continue without cytoplasmic constriction so that plasmodia containing from two to thirty nuclei are produced. Such a plasmodium is elongated and the nuclei are placed lengthwise. The plasmodia now become rounded. Each nucleus seems to divide once more (?) and the plasmodium breaks up into individuals each possessing two nuclei. From these, zygotes are formed (Debaisieux thinks that the vacuole-cells of Weissenberg are the zygotes). These sporonts are found in the vacuole occupied previously by the plasmodium. Whether or not the vacuole has an inner membrane cannot be determined. Each sporont divides into two sporoblasts by mitosis. The sporulation from this point on is similar to that of *G. mülleri* or *G. danilewskyi*. Occasionally the sporonts in the vacuoles may give rise to uninucleated individuals which contain an enormous nucleus and which probably transform into plasmodia by simultaneous multiple nuclear division.

Spore: Monies: Length 3 to 3.5μ, breadth 1.5μ. Often a clear space is seen in the spore.

Thélohan (1895): Ovoidal (Figs. 251-255), one end being attenuated. The spore membrane is apparently composed of two valves (Fig. 252). The polar filament is extruded under the influence of iodine water (Fig. 255). Length 4 to 4.5μ, breadth 3μ, length of the filament 30 to 35μ.

Stempell: The sporont that begins to sporulate, is oval (Fig. 258) and at first without a membrane but later becomes surrounded by one. The compact nucleus is often at one end close to the large vacuole. Later a smaller vacuole appears at the other extremity. The nucleus divides amitotically into four, the nuclear changes varying in different individuals. In mature spores four nuclei are to be found. As the nuclei divide, they become less deeply stainable, probably due to the elimination of chromatin substances. Two of the nuclei are those of the sporoplasm. The spore appears smaller in Canada balsam. The mature spore is oval (Fig. 256), with one end usually narrower than the other. Abnormal spores occur frequently. The smaller vacuole, often located at the side, shows frequently a portion of the filament, while in the large vacuole the coiled filament is sometimes noticeable when studied in glycerine after alcohol treatment (Fig. 257). The shell-valves were not made out. The filament penetrates through the sporoplasm; it is most probably surrounded by a thin membrane, and lies mostly in the large vacuole (Fig. 262). Filament extrusion takes place when the spore is immersed in iodine water for a long time or sometimes by fixation. The extruded filament is 150μ long, and has a small basal thickening; it is usually straight, and often accompanied by the spore-contents lying near the basal thickening. Length 6μ, breadth 2μ.

Weissenberg: Length 3.5μ, breadth 2.3μ. Structure similar to that of *G. hertwigi*.

Debaisieux: A small vacuole at the anterior end, in which two chromatin grains are found. In the posterior vacuole which is large, the filament is spirally coiled. Between these two vacuoles the biconcave sporoplasm is located. The filament was extruded under the action of glycerine.

GLUGEA DESTRUENS Thélohan 1892

[Figs. 265-267]

1892	*Glugea destruens*	Thélohan	1892 : 174
1895	*Glugea destruens*	Thélohan	1895 : 357

Habitat: The muscle of *Callionymus lyra* (a fish). Even the primitive fibrillae are invaded by the microsporidian and the infected muscle fibers undergo vitreous degeneration.

Locality: France (Concarneau, Roscoff).

Vegetative form: Protoplasmic mass with ectoplasm and endoplasm. Membraneless and not encysted.

Spore: Length 3 to 3.5μ, breadth 2μ (1892); length 3 to 3.5μ, breadth 2 to 2.5μ (1895) (Figs. 266, 267).

GLUGEA PUNCTIFERA Thélohan 1895

[Figs. 272, 273]

1895	*Glugea punctifera*	Thélohan	1895 : 218, 357

Habitat: The connective tissue of the eye-muscle of *Gadus pollachius*.

Locality: France (Concarneau).

Vegetative form: In the parasitic mass spores are found in the center and numerous fat globules stained black by osmic acid at the periphery (Fig. 272). Also diffused infiltration.

Spore: Ovoidal (Fig. 273). Identical with, but slightly larger than that of *G. anomala*. In the vacuole at the posterior end of the spore is present a small highly refringent globule whose nature is unknown. Length 4 to 5μ, breadth 3μ.

GLUGEA OVOIDEA Thélohan 1895

[Fig. 268]

1895	*Glugea ovoidea*	Thélohan	1895 : 357

Habitat: The liver of *Motella tricirrata* and *Cepola rubescens* (fish).

Locality: France (Roscoff, Marseille, Banyuls).

Vegetative form: White small bodies, 1 to 1.5 mm. in diameter.

Spore: Length 2.5μ, breadth 1.5μ (Fig. 268).

GLUGEA ACUTA Thélohan 1895

[Fig. 269]

1895	*Glugea acuta*	Thélohan	1895 : 358

Habitat: The connective tissue of the air-bladder muscle of *Syngnathus acus* and *Nerophis* (*Entelurus*) *aequoreus*. The microsporidian occurred in the host tissue in which a myxosporidian, *Chloromyxum quadratum*, was found.

Locality: France (Roscoff, Marseille, Concarneau).

Vegetative form: Tumors were observed; they were more elongated and voluminous than those of *Chloromyxum quadratum*.

Spore: Ovoidal; one end greatly rounded, the other highly pointed (Fig. 269). Iodine water brings out the polar capsule. No extrusion of polar filament was effected either by iodine water or by ether. Length 5μ, breadth 3 to 3.5μ.

GLUGEA CORDIS Thélohan 1895

[Fig. 270]

1895	*Glugea cordis*	Thélohan	1895 : 359

Habitat: Connective tissue and probably muscle of the heart of *Clupea pilchardus* (*Alosa sardina*). The parasite appeared as irregular spots white in color, covering the surface of the ventricle.

Locality: France (Marseille).

Spore: Ovoidal; smaller end is greatly pointed (Fig. 270). The polar capsule is distinctly visible when treated with nitric acid. Length 3 to 3.5μ, breadth 2μ.

GLUGEA DEPRESSA Thélohan 1895

[Fig. 271]

1895	*Glugea depressa*	Thélohan	1895 : 360

Habitat: Liver of *Coris julis* (*Julis vulgaris*). The microsporidian forms small white spots on the surface of the infected liver.

Locality: France (Marseille).

Spore: Elongated ovoidal (Fig. 271). A refringent globule, the polar capsule (?), is present at the smaller end. Length 4.5 to 5μ, breadth 1.5 to 2μ.

GLUGEA DANILEWSKYI Pfeiffer 1895

[Figs. 274-295; Textfigs. F, G]

1891	Microsporidie	Danilewsky	1891 : 9
1891	Microsporidien	Pfeiffer	1891 : 102, 103
1895	*Glugea danilewskyi*	Pfeiffer	1895 : 45, 73
1919	*Glugea danilewskyi*	Debaisieux	1919 : 158-161, 164-175
1922	*Glugea danilewskyi* (?)	Guyénot and Naville	1922a : 1-61

Habitat: The muscles of *Rana temporaria*, *Emys orbicularis* (*E. lutaria*, *Cistudo europaea*) and *Tropidonotus natrix*. Also parenchyma, intestinal cells, reproductive glands, excretory tubes of a trematode parasitic in the stomach (fixed in the mucosa) of the last named reptilian host. Infections probably occur also in *Lacerta* sp. and *Chalcides tridactylus* (*Seps chalcides*).

Danilewsky noticed that the microsporidian formed white and spindle-shaped bodies, 1 to 1.5 mm. long, in the muscle especially of the posterior part of the host body. It was found inside the sarcolemma and consisted of spores.

Debaisieux states that the infection was marked by the presence of small whitish cysts. The cysts are found especially in intercostal as well as spinal muscles and seem to exist in the tendon and intervertebral ligament. They are elongated in form, embedded in the muscle fibers and measure from 0.5 to 2 mm. in length. About ten to twenty cysts are found in a fairly heavily infected host. The tumor presents different aspects according to various stages of development, of which the author mentioned three. When the tumor is young, the spores are united into small masses covered with a hyaline membrane forming alveoli. Among the alveoli young stages are dispersed irregularly. The tumor is covered by the muscle and the muscle fibers are enclosed in the masses of spores as small bundles and in more or less atrophied condition. More or less developed connective tissue penetrates through the tumor. Some of the nuclei of the host tissue cells are isolated at the periphery or in the tumor. In a further advanced state, the tumor is a compact mass of the microsporidian spores, which is distinctly separated from the host tissue.

Guyénot and Naville stated that the microsporidian is found in encysted form either in the striated muscle fibers or connective tissue. They present diverse appearances in the two different locations. The authors also found that the microsporidian attacked a trematode living in the stomach of the reptile. Introduction of the microsporidian into the digestive tract (through mouth) of the native reptile followed by autopsies failed to show any cyst formation in the experimental animals.

Locality: Poland (Charkow), Germany, Italy (Verone, Bologne), Belgium (Louvain).

Vegetative form: Pfeiffer: The parasite forms a white striation in the muscle of *Rana temporaria*. It is 1 to 10 mm. long. Irregular vegetative forms assume small spherical granular masses about 1 cm. in diameter. The smallest are 3 to 4μ in length. Sporoblasts, 160 to 640μ in diameter, are surrounded by thin membrane. They contain 8 to 100 spores.

Debaisieux: The young stages are scattered among the secondary cysts, and are either isolated or grouped in islets of 20 to 30 individuals at different stages of development. The youngest individual is uninucleated and varies from 2 to 4μ in diameter (Textfig. F 1). This stage is ephemeral and rarely observed. As the body grows, the nucleus divides successively without a resting stage (Figs. 274-279). Nuclear division is in most cases rather simple. Frequently prior to division, the chromatic substance form two granules and each of these two divides simultaneously so that two granules are seen at each end. The division takes place successively. The nucleus is sometimes seen undergoing multiple division (Fig. 280). In some preparations, a promitotic division was noted. The daughter nuclei are at first localized in the center of the parasite, later becoming scattered. Thus the microsporidian reaches its plasmodial stage (Fig. 281). This stage seems to break up into smaller forms and produce younger stages already mentioned (Fig. 282). The contents of the plasmodial individual divide into numerous uninucleated bodies. The nucleus is voluminous with an irregularly contoured chromatic mass which often is clearly divided into two. When the nucleus divides, each daughter nucleus is composed of two chromatic masses. A stage with two nuclei closely approximated (Figs. 283-285) is thought to be the stage corresponding to the autogamous diplocaryon in *Thelohania varians*. The autogamous copula then divides once forming two sporoblasts (Fig. 286). The sporoblast becomes elongated (Figs. 287, 288) and its voluminous nucleus moves toward the posterior extremity (Fig. 291). The appearance at this stage is very variable. A deeply staining granule and a clear vacuole appear at the anterior end and another vacuole is also formed at the other extremity. The cytoplasm condenses itself toward the middle and peripheral part of the spore, assuming a girdle-like form (Figs. 292, 293).

Guyénot and Naville: In the intramuscular cysts, the youngest schizont is a small amoeboid form with two or four nuclei (Textfig. G 2) and measures 4 to 7μ in diameter. It grows and its nuclei divide amitotically. Finally it reaches 20μ in diameter and contain more than 20 nuclei. The cytoplasm then becomes vacuolated and the nuclei assume a more condensed appearance. These changes are followed by the division of the body into numerous uninucleated bodies (Textfig. G 5) which grow and repeat the development mentioned above (Textfig. G 6, 7). Often there are binucleated bodies comparable to Debaisieux's copulation stages, but they are in reality none other than the dividing forms. Schizogony is followed by gametogony. Some of the plasmodia possess rounded compact nuclei which become smaller later and the cytoplasm which becomes highly vacuolated (Textfig. G 9, 10). The plasmodium now divides into as many uninucleated spindle-shaped microgametes (Textfig. G 11) as there are nuclei. In a similar manner macrogametes are formed. They are either oval or attenuated at one end and more voluminous than the microgametes (Textfig. G 12-14). Anisogamy between the two gametes takes place though this process was not actually observed (Textfig. G 15). The sporulation occurs in groups or in pansporoblasts [although the authors speak about a pansporoblast, none of the figures given by them shows a typical pansporoblast membrane]. The latter consist of 16 to 60 or more spores. In the smears two types of sporulation were noted. The sporoblast with a large nucleus has deeply staining cytoplasm (Textfig. G 16-19). The other sporoblast with a small nucleus, possesses a feebly staining cytoplasm (Textfig. G 21-23). The former sporulation is considered as belonging to sexual cycle, the latter type as belonging to asexual or parthenogenetic development. In the intraconnective tissue cysts, the young stages are feebly staining small amoeboid bodies occupying the vacuoles of the large connective tissue cells. They develop directly into pansporoblasts with smaller nucleil They divide amitotically into sporoblasts which become isolated in the host tissue vacuoles and transform into spores.

Spore: Danilewsky: Oval. In mature spores, the central portion is more transparent than in younger forms in which the shell does not show the double contour. Length about 3 to 4μ.

Pfeiffer: Pyriform or oval. Length 3 to 4μ.

Debaisieux: A vacuole at each end (Figs. 292, 293). In the anterior vacuole a chromatic granule is seen in connection with a staining filament which forms a rather voluminous chromatic mass at the posterior part of the vacuole. The shell is undoubtedly a single envelope. The existence of a capsulogenous cell is doubtful and the polar capsule proper does not exist. The filament probably develops from the chromidial granules. In the posterior vacuole a granular nucleus is found. The filament was not extruded. Size varies greatly. Average dimensions: length 3 to 4μ, some large forms 6 to 7μ. Larger ones are rare.

Guyénot and Naville: Ovoidal. When the spore is treated with a weak solution of hydrochloric acid and stained with Heidenhain's iron hematoxylin and Bordeaux red, one can see the pyriform polar capsule at the slightly attenuated end and the binucleated sporoplasm near the rounded end (Fig. 295). Macrospores 4μ long; microspores 3μ long. The filament is extruded under the influence of the acid and measures 50 to 70μ in length. The spore membrane is a single piece.

GLUGEA MÜLLERI Pfeiffer 1895

[Figs. 296-313]

1895	*Glugea mülleri*	Pfeiffer	1895 : 21, 53, 175-182
1919	*Glugea mülleri*	Debaisieux	1919a : 161-175

Habitat: The muscle of *Gammarus pulex* and *G. locusta*.

Debaisieux states that the infected host was abundant. It showed white filaments, 0.5 to 2 mm. long, especially at the posterior part of the body. The host showed from one to ten parasitic masses. These masses remain separated from one another. The appearance of the tumor was similar to the early phases of that of *G. danilewskyi*. In general, the infiltration of the parasite in the muscular tissue is marked more distinctly than that in the latter form. The demarkation between healthy and infected tissues was vague. It is probable that no encystment occurs in the present microsporidian.

Locality: Germany and Belgium (Louvain, throughout the year).

Vegetative form: Debaisieux: The young stages (Fig. 296) are usually multinucleate, although uninucleated forms are sometimes found. In the dividing nucleus the daughter nuclei, each containing two granules, are united by a filament. The division is probably karyokinesis. The protoplasm of the plasmodium breaks up into simple elements, each with two nuclei surrounded by alveoli (Fig. 300). Later the two nuclei undergo fusion and the zygote is formed (Fig. 301). These binucleated individuals (autogamous diplocarya) are smaller in number and their development is more difficult to observe than those in *G. danilewskyi*. Each zygote, the sporont, gives rise to two sporoblasts (Figs. 302-304). This division is marked by distinctness in appearances in which two connecting threads (Fig. 303) are sometimes seen. As in the case of *G. danilewskyi*, the incompletely separated sporoblasts regenerate the young stages by repeated nuclear divisions. The sporoblasts are of ovoidal form. Its nucleus moves toward the posterior end, while a chromatin granule appears in a vacuole at the anterior end (Fig. 305). This granule stains red either by safranin or by eosin-azur. Whether or not this is a nucleus, remains unsettled. The anterior vacuole becomes larger, the granule increases in size and its form changes from an irregular to a dumb-bell form, one end being attached to the anterior tip and the direction assuming an eccentric course (Figs. 308, 311). The posterior nucleus is expanded considerably and the round end of the spore becomes occupied by a vast vacuole which is separated from the membrane. The chromatic portion of the nucleus reduced into a small irregular lump, is found either at the posterior tip or at the side of the posterior vacuole (Figs. 310-312).

Spore: Debaisieux: Ovoidal; a vacuole at each end. The sporoplasm is at the middle part of the spore and is biconcave in shape; through it passes a small canal which joins the two vacuoles. The nucleus is at first seen at the posterior part of the spore but migrates into the sporoplasm as the spore becomes mature. The spore membrane is undoubtedly a single piece. The existence of the capsulogenous nucleus is doubtful. No polar capsule exists. In the posterior vacuole, the filament is coiled two or three times and one end is connected with the canal through the sporoplasm. No filament extrusion was noted. The size differs considerably without any distinction between microspore and macrospore. Length 5 to 6μ, breadth 2 to 3μ.

Remarks: This species should be compared with *Thelohania mülleri* and *T. giraudi*.

GLUGEA LAVERANI Caullery et Mesnil 1899

1899	*Glugea laverani*	Caullery and Mesnil	1899 : 791-792

Habitat: The body cavity and tissue of *Scoloplos mülleri*; also the epidermis and its derivative (nervous system) and the body cavity of *Scolelepis fuliginosa* (both Polychaeta). Very rare. When the parasites occur in the host body cavity, they are surrounded by the phagocytes.

Locality: France (Saint-Martin, near the Cape of Hague; but not at Wimeraux nor at other places).

Vegetative form: Elongated amoeboid. Form very irregularly and greatly variable. Loaded with the spores. Often spherical in form. The ectoplasm is not distinctly differentiated, but the external zone does not contain spores. The nuclei are vesicular and each shows a karyosome. The sporoblasts are difficult to recognize.

Spore: Ellipsoidal, with a clear vacuole at one extremity. Length 4 to 4.5μ, breadth 1.5 to 2μ.

GLUGEA STEPHANI (Hagenmuller 1899) Woodcock 1904

[Figs. 314-316; 759]

1899	*Nosema stephani*	Hagenmuller	1899 : 836-839
1901	Sporozoan	Johnstone	1901 : 184-187
1901	Protozoan	Linton	1901 : 485
1904	*Glugea stephani*	Woodcock	1904 : 46-59

Habitat: In the gut-wall, liver and mesentery of *Pleuronectes flesus* (*Flesus passer*), *P. platessa* and *Pseudopleuronectes americanus.*

Hagenmuller observed 18 infected host fish out of 30 examined. Johnstone examined two specimens, both female, of the second host caught in October. The fish appeared normal; the liver, kidney, intestine and other organs having their usual situations. But the greater portion of the intestine of one of the fish, from the pylorus to within about 1.5 inches of the anus was thickened and had the appearance of a ripe ovary. On cutting open a part of the gut it was seen to have a much reduced lumen. The wall was 3 to 4 mm. thick. Its inner surface was thrown out into close set and deep longitudinal folds pursuing a zigzag course. The narrowness of the lumen was due to whitish colored round bodies projecting into it. The stomach was normal in form and relations except that its wall seemed thinner than usual. Almost the entire post-pyloric part of the intestine of the second specimen was modified in precisely the same way as in the other.

Linton examined two small specimens of the third host from Katama Bay in August. The intestine was chalky white in color and the wall of the intestine of one fish throughout almost the entire length and of the other for a short distance was completely covered with the "sporocysts." Mavor found out that about 50 per cent. of the same host fish in Woods Hole region were infected with the microsporidian, in the summer and winter of 1910.

Woodcock noticed the seat of infection in the second host was the gutwall, from esophagus to rectum, in the muscular and connective tissue (sub-mucosa). The parasite often occurs in the cyst form, projecting into the coelomic cavity (Fig. 759). They are also found under the peritoneum infecting the liver, and in the mesenterial folds in which the blood vessels run. Other organs such as kidney, heart, were free from the microsporidian.

Locality: France, England and the United States.

Vegetative form: Hagenmuller: The microsporidian invades the host tissue either in cyst form or in a state of diffuse infiltration. The cyst appears to the naked eye as a small white ovoidal or spherical body, not exceeding 1 mm. in diameter, usually 0.5 mm. or less. It is covered with a membrane composed of the host tissue. Adult cysts contain numerous spores and residual granular masses. Younger cysts contain sporoblasts which are groups of spores surrounded by fibrous envelope.

Johnstone: The cysts, perfectly spherical in fresh state and with an average diameter of about 600μ, fill up the intestinal wall (Fig. 314). Between the cysts there are to be found a few connective tissue fibers. The cyst is covered with a rather thick membrane and is filled with a vast number of spores.

Linton: Irregular, elliptical or spherical in form, the cysts varied in size, a few reaching 1 mm. in diameter.

Woodcock: The parasite occurs either in cyst form or in a state of diffuse infiltration. The cysts (Fig. 315) are well-defined, and sharply limited. They are surrounded by delicate layers of the host connective tissue. The peripheral portion may be called the ectorind (a modified ectoplasm). Internal to this there is a layer without any indication of spore formation, but identical with the endoplasm into which it passes. The endoplasm is a comparatively narrow layer, as by far the greater part of the cyst consists of an immense number of spores. It is the seat of spore formation and contains a great number of pansporoblasts in various stages of development, from little uninucleated forms to large forms which are practically clumps of sporoblasts. Scattered about in the endoplasm, and also occasionally met with in the central mass, are large sometimes drawn-out nuclei with fragmented chromatin and a distinct nucleolus, their origin and significance being unknown. Passing inwards one finds clusters of ripe spores, and these soon almost entirely replace the endoplasm and run together to form the central mass of spores. Often tongues of endoplasm projecting towards the center were seen and in some sections they appeared as isolated patches. Pseudocysts are formed by union of small forms occurring in the state of diffuse infiltration. They arise probably as a result of an endeavor on the part of the host to limit the infiltrated area by the arrangement of hypertrophied connective tissue in concentric layers round the center of infection. The development of the spore could not be followed.

Spore: Hagenmuller: Undescribed.

Johnstone: Oval. Maximum diameter about 5μ (Fig. 316).

Linton: Oblong-ovate. Length 3μ, breadth 1.5μ.

Woodcock: Oblong-ovate. Filament extrusion was not attempted as the author did not have fresh spores. Two vacuoles at ends. In one or two instances, a faint longitudinal sutural line marking the junction of the two shell-valves was seen. Polar capsule was not seen. One of the vacuoles is well marked, and invariably contains a small, rounded, deeply-staining granule; whilst the other seems to vary in size and is not always obvious. The former is probably the polar capsule. The other vacuole tends to increase in size with the ripening of the spore and may assist in separating the valves and liberating the sporoplasm. The nucleus in the sporoplasm is at first single and round, but later divides into two passing through a horseshoe-form stage. Three nuclei were sometimes noticed in the sporoplasm.

GLUGEA SHIPLEI Drew 1910
[Fig. 317]

1910	*Glugea shiplei*	Drew	1910 . 55-57

Habitat: In the skeletal, stomachal and intestinal muscles of *Gadus luscus*. The fish, 10 cm. long, was seen to have a number of oval transparent cysts irregularly scattered. The superficial cysts were not connected with the skin, and none had ruptured externally. No trace of cysts could be found in any other tissue.

Locality: England (Plymouth).

Vegetative form: Cysts, the largest of which reached 5 mm. by 3 mm. with one or more opaque white spots seen with naked eye, were surrounded by a very thin and transparent membrane. The intramuscular fibers are the seat of infection. The slightly granular protoplasm contained a large number of nuclei. The developing pansporoblasts appear to migrate towards one or more centers in the protoplasm, with the result that one or more spherical masses of densely packed spores are formed. Besides, a few large elongated nuclei of vegetative nature occur.

Spore: Pyriform (Fig. 317). A small aniodinophilous vacuole is present at the broader end, and a very minute body at the apex probably represents the polar capsule. The filament could not be distinguished. Attempts to cause filament extrusion by treating with reagents failed.

GLUGEA HERTWIGI Weissenberg 1911
[Figs. 318-335, 767]

1911	*Glugea hertwigi*	Weissenberg	1911b : 344, 348-351
1913	*Glugea hertwigi*	Weissenberg	1913 : 89-163
1915	*Glugea stephani* (?)	Mavor	1915 : 36
1921	*Glugea* sp.	Schrader	1921 : 151-153

Habitat: In *Osmerus eperlanus* (Weissenberg) and in fresh and salt water forms of *O. mordax* (Schrader). Weissenberg notes that the microsporidian invades the subcutaneous connective tissue, substantia propria of the cornea, gut wall, stomach, liver, connective tissue of the gonads and body cavity in the first host. The parasite is responsible for the cysts which vary greatly in size. About 1 to 2 per cent. of one year old fish examined were infected. The transparency of the body of the fish, even in a specimen 10 cm. long, made the macroscopic detection of the cysts easy. One hundred fish were studied, of which 14 were sectioned.

Schrader mentions that the intestine is apparently the primary seat of the parasite (Fig. 331). Affected fishes are characterized by the appearance of numerous cysts in the viscera, generally all the cysts in a fish being approximately of the same size (Fig. 767). The cysts reach up to 3 mm. in diameter. At the beginning the cysts are located in the mucosa, below the epithelium of the villi, but as they grow they push through the muscular coat of the intestine and then come to lie immediately under the peritoneum. The entire length of the intestine from below the stomach to within a short distance of the anus may be invaded by cysts. Cysts also occur in the liver and the gonads, but not in the stomach, kidney or heart.

Mavor remarked that he saw frequently *Osmerus mordax* from Woods Hole infected by a microsporidian which he held as *G. stephani*. This may be identical with the form observed by Schrader.

Locality: Germany (Lietzew on Rügen) and the United States (New Hampshire, Maine coast, Woods Hole).

Vegetative form: Weissenberg: When the cysts lie closely together, the surface becomes flattened. The cyst has a distinct membrane and is surrounded by layers of connective tissue of the host, in which capillary networks are formed. The thickness of the cyst wall is about 2μ in a cyst of 2 mm. in diameter. The membrane is stained somewhat intensively with nuclear stains and is in direct contact with the outer layer of the cyst. The cytoplasm is solid around the periphery, contains fluid vacuoles towards the interior and is replaced by a clear space in the center. Young spores and developing stages are found in the vacuoles in the periphery, while the mature spores fill the central portion. The larger the cyst, the narrower the peripheral region. In an old cyst, the peripheral protoplasmic portion is entirely wanting. The structure of the protoplasm of the cyst varies from finely granular to reticular. Large vegetative nuclei of 20μ or more in diameter and of a vesicular type with a distinct membrane and one or more large nucleoli stained red by Biondi's method, are found near the periphery. The nuclei seem to multiply. Often the migratory cells of the host are observed in the cyst and spores are undoubtedly taken inside of these cells by phagocytosis. The youngest stage found in the cyst is a primary cell which after elongation of its body develops into a primary cylinder, though the formation was seldom observed. The primary cylinders, 7μ in length, are seen in large groups in the peripheral layer as well as in the central portion of the cyst. There are binucleate primary cylinders, up to 9.4μ in length, which are probably formed from the uninucleate forms. No autogamous changes occur in this stage. These cylinders grow with the nuclear divisions. As an example, a cylinder with eight nuclei in four pairs is 12 to 17μ long. The nuclei divide further and the body grows, reaching 20 to 30μ in length with 10 nuclei arranged in pairs, though sometimes the latter are arranged irregularly in two rows (the cylinder is then 18μ by 4μ). The maximum number of the nuclei in a cylinder is 32 or 42. Weissenberg distinguished primary and secondary cylinders among these according to the number of nuclei present. A fluid vacuole is formed around the secondary cylinder and the latter breaks up successively or simultaneously into as many cells as the nuclei after becoming rounded. These vacuole cells are spherical or polyhedral bodies, each with a compact spherical nucleus, and are the mother cells of the sporoblasts. They are 3 to 3.5μ in diameter with a nucleus of 1.4μ in diameter. The latter divides into two, the connecting thread remaining for some time. The vacuole cell divides and forms two sporoblasts. These are spherical at first, then become elongated (Fig. 318) and assume the oval form of the spores (Figs. 322 to 330). The nucleus is at the narrow end. It is spherical and vesicular. At the opposite end a thickening which later becomes the filament, appears. The nucleus now gradually moves into the middle of the young spore and a large vacuole appears at the broad end.

Schrader: As in other species of Glugea, sporonts, sporoblasts, and ripe spores may all be found in a single cyst, with the earliest stages near the periphery. Sporulation seems to follow the same lines as described for *G. anomala* by Stempell and Awerinzew and Fermor.

Spore: Weissenberg: Elongated pyriform. Fresh spores show a large vacuole at the broad end (Fig. 322). By adding dilute acetic acid, a smaller vacuole is made visible at the opposite end, which is the result of artefact (Fig. 323). In fish, laid aside for a few hours, some spores extrude the filaments. Filament extrusion takes place when dilute iodine solution is added to spore emulsions kept in a moist chamber. In large vacuole, metachromatic granules are often recognized (Figs. 325-330). Length 4.6 to 5.4μ, breadth 2.3μ, length of the filament 100μ.

Schrader: Length 4 to 4.5μ, breadth 2 to 2.5μ (Figs. 332-335).

Remarks: The European and American forms are placed here in one species.

Genus PEREZIA Léger et Duboscq 1909

The characters of the genus are described on page 66.

Type species: *P. lankesteriae* Léger et Duboscq 1909.

PEREZIA LANKESTERIAE Léger et Duboscq 1909

[Figs. 336, 337]

1909	*Perezia lankesteriae*	Léger and Duboscq	1919 : LXXXIX-XCIV

Habitat: The cytoplasm of *Lankesteria ascidiae*, a gregarine, parasitic in the intestine of *Ciona intestinalis* (Tunicata). The tissues of Ciona were not attacked and only those gregarines that were free in the intestinal lumen were subjected to the microsporidian infection, all the intracellular forms being free from the parasite. Thus the microsporidian is exclusively gregarinophilous.

The infected gregarines are the cephalonts or sporonts, nearing the adult stage. The spores are ordinarily scattered throughout the host body, and are not found in groups in dense masses such as is the case with *Nosema frenzelinae* (page 89). The host nucleus is not affected even when the infection is very heavy. The cytoplasm does not show any changes as a result of the infection, although occasionally hyaline strands enclosing spores are seen. Whether or not the infected gregarines can encyst is unknown, but the condition is doubtlessly like that of *N. frenzelinae.* Perhaps as in the latter's case, the infection may bring about the death of one or both of the conjugants.

Locality: France (Cette).

Vegetative form: The youngest stage is a small uninucleated ovoidal mass which grows and multiplies (Fig. 336). The nuclear division is mitotic in which the axial chromosme is clearly noticeable. The cytoplasmic constriction does not accompany the nuclear division, resulting in the formation of plasmodia. These may contain 10 or 12 nuclei which measure 3μ and are grouped in the center. They finally break up into uninucleated bodies, the sporonts. Each sporont is elongated, 6μ long, and divides into two sporoblasts, each of which gives rise to a spore.

Spore: Ovoidal, 2.5μ long. The filament extrusion was not noted. The two spores become separated from each other by the movements of the host gregarine. Sometimes abnormal spores are found; some are large, elongated (7 to 8μ long), isolated and possess numerous chromatic elements. They are without doubt the products of monosporous sporulation.

PEREZIA MESNILI Paillot 1918

[Figs. 338-344]

1918	*Perezia mesnili*	Paillot	1918 : 66-68

Habitat: Silk-glands and Malpighian tubules of the larvae of *Pieris brassicae* (Lepidoptera). The incidence of infection was not high and the microsporidian does not seem to have a wide distribution.

Locality: France (Sathonay-Rillieux; but no infection was noted in the host insects studied at Saint-Genis-Laval).

Vegetative form: Two types of schizogony are noted. 1) Binary division (Figs. 338, 339). The schizont is rounded. It possesses two nuclei located generally in the center of a vacuole and arranged like two coffee berries (from which Paillot concludes that the division is a mitosis). Sometimes the binucleate forms remain undivided and form chainforms composed of up to four individuals. 2) Multiple division. The schizonts contain 4, 6 or 8 nuclei which are paired (Fig. 340). The size of the body is not proportionate to the number of the nuclei. At the end of schizogony, an elongated binucleated sporont is formed (Fig. 341). Each nucleus divides once and two daughter nuclei move towards the opposite poles (Figs. 341, 342). The cytoplasm becomes vacuolated and is stained less intensively. This is the sporont which divided into two sporoblasts and measures 8μ in length. The isolated sporoblast which is ready for sporulation possesses two compact nuclei. In some cases one finds besides a small nucleolus which migrates toward one of the ends of the sporoblast and constitutes the base of the polar filament (Fig. 343). The filament extends toward the opposite pole and becomes spirally coiled twice (Fig. 343).

Spore: Elongated ovoidal; form variable (Fig. 344). Macrospores and microspores. The filament extrusion was unnoticed, but appears to be 18 to 20μ long. Spore: length 3.4μ, breadth 1.5 to 2μ.

PEREZIA LEGERI Paillot 1918
[Figs. 345-356]

1918	*Perezia legeri*	Paillot	1918a : 187-189

Habitat: The fat body and giant cells in the blood of larval *Pieris brassicae*. In the case of general infection (rare), the spores are found in all tissues. The giant cells, observed very often in the blood, show variable dimensions, reaching 150μ in diameter in which case, it appears as a small white granule to the unaided eye. When the host cell containing the spores is examined in fresh state, it may be mistaken for a floating cyst. Since several intermediate stages are noted in the blood of certain host larvae, they most probably arise from ordinary elements of the blood. The cytoplasm is stained deeply, making the nucleus hardly visible and the cell wall is compact and refractive and resembles a cyst membrane under low magnification. When the hyaline cytoplasm transudes outwardly, it presents rounded pseudopdia-like projections. The nucleus is large and the chromatin appears filamentous. Under what influence this hypertrophy of the blood cell occurs, is not known.

Locality: France (Sathonay-Rillieux).

Vegetative form: It differs little from that of *P. mesnili*. Multiplication by binary and multiple fissions (Figs. 345 to 350). The plasmodia are large and irregular in form (Fig. 347). The nuclei are always found in pairs. At the end of schizogony, the binucleated element becomes elongated and less deeply stainable. This is the sporont. Two sporoblasts are formed from the latter, each developing into a spore.

Spore: Elongated oval (Fig. 355), 4 to 5μ in length (fresh). At the more rounded end a vacuole is located, which was not seen in *P. mesnili*. Extruded filaments measure 30 to 40μ long (Fig. 356). The filament is extruded from the rounded end. Two nuclei are seen in the spore. In some of the infected fat bodies (smears), Paillot saw the binucleated sporoplasm leaving the spore at its side (Fig. 354).

Genus GURLEYA Doflein 1898

The characters of the genus are described on page 66.

Type species: *G. tetraspora* Doflein 1898.

GURLEYA TETRASPORA Doflein 1898
[Figs. 357-364]

1898	*Gurleya tetraspora*	Doflein	1898 : 291

Habitat: The hypodermal tissues of *Daphnia maxima* (Entomostraca).

The microsporidian infection was noticeable because of the opaque appearance of the host body.

Locality: Germany (in the vicinity of Munich).

Vegetative form: Smears contained numbers of pansporoblasts at various stages of development and fully formed spores (Figs. 357-359).

Spore: Oval; one end pointed, the other rounded (Figs. 360-364). The spore membrane with fine striations (ridges) (Figs. 358-360). A large clear vacuole at the broad end. Spore was not studied in fresh state. Dimensions not given.

GURLEYA LEGERI Hesse 1903
[Figs. 365-369]

1903	*Gurleya legeri*	Hesse	1903a : 495-496
1904	*Gurleya legeri*	Hesse	1904 : 1-3
1911	*Gurleya legeri*	Mackinnon	1911 : 34-36

Habitat: In the fat body, muscle and connective tissue of nymphs of *Ephemerella ignita* (Hesse) and in the fat body of larval caddis-fly (Mackinnon).

Hesse found four per cent. of the host animals studied by him were infected by the microsporidian, while Mackinnon noted only three parasitized hosts out of two hundred individuals examined. According to the latter author, the parasitised larva moves about sluggishly, and has a swollen congested appearance. The infected fat body is chalky white.

Locality: France (near Haute-Saône), September and October; and England (Aberdeen), late summer.

Vegetative form: Hesse: At the end of evolution, pansporoblasts with macrospores and with microspores appear, the latter predominating in number over the former. Pansporoblasts with microspores are ellipsoidal (11μ by 5μ) and the spores are arranged in two rows, rarely side by side in one row (8.5μ by 5μ). Pansporoblasts with macrospores are spherical (5 to 8μ in diameter) or slightly ovoidal (8μ by 6μ). Three or often only two spores are found in a pansporoblast. Some contain both of the spores: one macrospore and one or two microspores.

Mackinnon: Early stages were unobserved. Pansporoblasts with four spores, sometimes with three, are oval to round in form and measure from 8μ by 5μ to 11μ by 6μ. The spores are arranged therein in two superimposed rows: sometimes the narrow ends all point the same way, more often the narrow ends of the upper row lie above the broad ends of the row beneath (Figs. 365 to 369).

Spore: Hesse: Ovoidal. Sulphuric acid causes filament extrusion in microspores, while the macrospores do not show any evidence of having a filament. Macrospores 5 to 6μ long by 3 to 4μ broad. Microspores 4 to 5μ long by 2.5μ broad; the filament 24 to 25μ in length.

Mackinnon: Macrospores and microspores are not distinguished. Filament extrusion was noted with sulphuric acid and the filament is 25μ long (nearly). The spores are pear-shaped: length 4 to 5μ, breadth 2.5 to 3μ.

GURLEYA FRANCOTTEI Léger et Duboscq 1909
[Figs. 370-382, 776]

1909 *Gurleya francottei* Léger and Duboscq 1909c : 894-898

Habitat: Epithelial cells of the midgut of the larva of *Ptychoptera contaminata*. The microsporidian occupies the epithelial cells which are free from *Pileocephalus striatus*, a gregarine, occurring in the same host organ. All the epithelial cells in the limited region were infected and filled with the stages of parasite. The host cell becomes enlarged to twice the normal size and the cytoplasm disappears. The host nucleus also undergoes hypertrophy and shows chromatolysis and karyolysis. The cell membrane, however, remains in spite of the enormous multiplication of the parasite in the cytoplasm (Fig. 776). The gregarines are free from the microsporidian infection.

Locality: Belgium.

Vegetative form: The schizonts (Fig. 370) are about 4μ in diameter, each containing a round nucleus. The nucleus is composed of a nucleolus or karyosome, some fine chromatic grains in the periphery and a centrosome which can be seen more distinctly when in division. The nucleus divides mitotically (Fig. 372 to 375). In anaphase there is an axial chromosome uniting two groups of chromosomes (Fig. 373). The schizonts always assume a spherical form due to the presence of a fairly distinct membrane. Associated with them are found the pansporoblasts which are much larger (6μ) with a large nucleus containing fine grains scattered on chromatic network (Fig. 377). Whether the pansporoblast is formed by the copulation of two gametes (as in *Thelohania giardi* observed by Mercier) or not, was not determined, although forms with two unequal nuclei were sometimes recognized (Fig. 376). The nucleus of the sporont divides twice successively producing four nuclei and the cytoplasm breaking up into four parts at the same time (Figs. 378-381). Each sporoblast becomes a spore but the change was difficult to follow. Four pyriform spores become united with their rounded ends in a form of a cross (Fig. 382). When the sporulation is completed the four spores are destitute of a common membrane as in other species of the genus and the tetrasporous character cannot be seen (Fig. 776).

Spore: Pyriform. Length 3μ. Stained deeply.

GURLEYA RICHARDI Cépède 1911

[Figs. 383-392, 753]

1911	*Gurleya richardi*	Cépède	1911 : 29-32

Habitat: In *Diaptomus castor* (female). The microsporidian occupied the entire posterior region (three segments) of the cephalothorax which appeared chalky white in color (Fig. 753).

Locality: France (Wimeraux-Ambleteuse).

Vegetative form: Only advanced stages were seen. The schizonts (Figs. 383 to 386) are subcircular in form and measure 4.22μ by 4μ to 6μ by 3.5μ or 6μ by 4.5μ. Its cytoplasm is finely granulated. The spores are disposed within the common membrane. The arrangement of the spores depends upon the form of the pansporoblast. The latter measures 6μ in diameter, 9.4μ by 4.5μ, 7.5μ by 4.25μ, 7.5μ by 4.5μ. Those with macrospores (Figs. 390, 391) are slightly larger than the pansporoblasts with microspores (Figs. 387 to 389).

Spore: Macrospores are 5.5 to 6μ long by 2.8μ broad. Microspores are 4 to 4.5μ long and the length of the filament is 45μ. Immersing the spore for ten minutes in physiological solution causes the filament extrusion (Fig. 392).

Genus THELOHANIA Henneguy 1892

The characters of the genus are described on page 67.

Type species: *T. giardi* Henneguy 1892.

THELOHANIA GIARDI Henneguy 1892

[Figs. 393-434; Textfigs. B 1, C]

1892	*Thelohania giardi*	Henneguy and Thélohan	1892a : 626-631
1892	*Thelohania giardi*	Thélohan	1892 : 174
1894	*Thelohania giardi*	Gurley	1894 : 201-205
1895	*Thelohania giardi*	Thélohan	1895 : 362
1908	*Thelohania giardi*	Mercier	1908 : 34-38
1909	*Thelohania giardi*	Mercier	1909 : 30-43

Habitat: The muscle of *Crangon vulgaris* (crustacean).

Henneguy and Thélohan examined a host specimen from Wimereux-sur-Mer. The appearance of the infected host was similar to that of the host of

T. octospora except that the change was less striking compared with the latter species by reason of the lesser transparency and the pronounced tegumentary pigmentation of the present host. There probably occurs the so-called castration by the parasite. The muscles of *Caradina desmuresti* which were fed for 71 days on the infected Crangon muscles, were free from the infection, although in their excrement, a large number of empty spores with separated shell-valves were observed.

Locality: France (Boulogne, other places not mentioned).

Vegetative form: Henneguy and Thélohan: The sporonts, "vesicles," are spherical and measure about 14μ in diameter. The membrane thin and the cytoplasm highly transparent and granulated. The nucleus is large and often visible in the fresh state. It is located in the center. The nucleus at first presents a typical resting stage, composed of a distinct membrane and a karyosome (one large grain) or of a number of chromatic granules (Fig. 393). The nucleus divides mitotically (Figs. 394, 395). In this division, the chromatin assumes a thread-like form, the membrane disappears, the chromatic threads become located in a very distinct equatorial plate which is doubled and the division of the nucleus into two daughter nuclei is finally completed. The nuclei divide twice more and form eight nuclei, around each of which the cytoplasm becomes condensed (Fig. 397). Thus eight sporoblasts are formed. They are arranged without order and have usually a truncate-pyramidal form, though the latter varies according to the arrangement inside the membrane. Each sporoblast develops into a single spore. The sporont membrane remains throughout the entire changes and often shows two marked thickenings which are visible in optical section (Fig. 400). Sporulation was difficult to follow. Often a clear round space into which a small protoplasmic button projected, was seen in the sporoblast; this may have been the developing polar capsule (Fig. 399). The youngest stage was uninucleated.

Thélohan (1895): The pansporoblasts are spherical, 14μ in diameter and each contains eight spores. The envelope possesses two clear spots.

Mercier (1909): The microsporidian is found in the muscle fiber. The schizonts are 2 to 7μ in diameter and their nucleus is very characteristic in the chromatin being arranged in a stellate form with many branches. The schizonts multiply by binary fission or often by multiple division. Some schizonts were noted which appeared undergoing degeneration; they were characterized by their size, 2 to 4μ in diameter, and by a compact chromatic mass found in them. The schizogony continues until the schizonts penetrate through the interior of the fiber. The schizonts now become the gametes. The gametes are all similar (Fig. 410). They are generally ovoidal and are about 3μ in largest diameter. The nucleus is a small vesicular body with chromatic granules attached to the nuclear membrane. These gametes are the final products of schizogony. Two of them fuse into one: the chromatin of the two nuclei breaks up into fine granules; at the same time the nuclear membrane disappears and the fusion of the two nuclear substances follows (Figs. 411-414). Thus by an isogamous copulation a copula, the sporont, is formed. The young sporont is a round body, 5 to 7μ in diameter and possesses a distinct membrane and a large nucleus situated in the center. The chromatic granules, the real chromidia, become detached from the syncaryon (Figs. 414, 415), migrate into the cytoplasm, and assemble finally under the membrane, meanwhile the nucleus exhibits a characteristic appearance. Now the nucleus divides and the process is intermediate between mitosis and amitosis. During nuclear division, the chromatin becomes grouped in two places under the membrane, later forming two masses (Figs. 416 to 419). Each of these divides twice more, thus forming eight sporoblasts (Fig. 424). Two residual chromatic masses remain inside the pansporoblast. After a resting period the nucleus of the sporoblast assumes a vesicular appearance and the chromatin becomes separated into four granular masses scattered under the nuclear membrane (Fig. 426). The nucleus divides into two equal daughter nuclei (a and b). One of them (a) divides again in two (a′ and a″), of which one divides once more. The nucleus (b) divides equally into two. Five nuclei are thus formed (Figs. 427-429). These divisions may be delayed or may take place rapidly. During these nuclear changes, there appear in the sporoblast two cytoplasmic laminae in crescent form, each with a nucleus which was derived from one nucleus (a′) by division, which later develop into the spore membrane (Figs. 430-432). The remaining three nuclei remain in the central part, each having a central karyosome (Fig. 430). A vacuole becomes differentiated in the region between the valve-cell nuclei and the remaining ones (Fig. 431). One nucleus (a″) becomes attached to the margin of the vacuole. This is the polar capsule, in which the filament is formed. The coiled filament may be noticeable (Fig. 433). Later vacuoles appear at the ends, the sporoplasm taking the central position (Fig. 434). In some spores, each of the nuclei of the sporoplasm may divide into two which are often unseparated and remain in pairs (Fig. 434).

Spore: Henneguy and Thélohan: Pyriform, anterior end more pointed, the other end rounded (Fig. 401). Highly refringent. The spore membrane shows very fine longitudinal striations. Whether or not the spore membrane is composed of two valves could not be determined. The polar capsule was not seen; the filament was extruded by hydrochloric and nitric acid , but not by iodine, potassium or sodium nitrate, glycerine, heat, acetic acid, formic acid or ether. It is well stained by aniline dyes, especially violet 5B. The sporoplasm when stained, showed two or three colored granules in the vacuole which is aniodinophilous. Length 5 to 6μ, length of polar filament 15 to 20μ.

Thélohan: Ovoidal; anterior end greatly pointed. The spore membrane is longitudinally striated (Fig. 403). The existence of two shell-valves was clearly observed. The filament of some spores was extruded by means of nitric acid (Fig. 404), while ether did not affect the extrusion. Length 5 to 6μ, length of the filament 15 to 20μ.

Mercier: Elongated oval. A vacuole at each end. Shell composed of two valves. Striation unobserved. The polar capsule is pyriform and is independent of the vacuoles. The coiled filament is visible. The sporoplasm is in the central portion of the spore. It usually has two nuclei, but often four. Dimensions not given (Textfig. B 1).

THELOHANIA ACUTA (Moniez 1887) Schröder 1914

[Figs. 435-439]

1887	*Microsporidia acuta*	Moniez	1887 : 185 1887a : 1314
1914	*Thelohania acuta*	Schröder	1914 : 324-327

Habitat: The fat body of *Cyclops viridis* (*C. gigas*) and *Daphnia pulex*.

Locality: France (Lille) and Germany (Landstuhl).

Vegetative form: Schröder: Each pansporoblast develops into eight spores which are covered by a common membrane (Fig. 435). No residual protoplasm of the host tissue between the pansporoblasts. The growth of the pansporoblasts causes the hypertrophy of the nucleus of the fat body.

Spore: Moniez: Pyriform, one end highly pointed. Length 5μ, breadth about 2μ.

Schröder: Elongated pyriform; circular in cross section. Anterior tip somewhat truncated. Fresh spores show a large clear pyriform space, the polar capsule, in the anterior portion, and a spherical vacuole at the posterior end. The sporoplasm is situated in between. The nucleus of the latter is variable in form, number and position (Figs. 436-439). The polar capsule, especially its posterior margin, was made clear by Mallory's staining. Length 5μ, breadth 2μ.

Remarks: Schröder thinks the species is distinguished from *T. virgula* (Moniez) by the arrangement of the spores; i.e., in the latter they are arranged in a stellate form which is never found in the present species.

THELOHANIA VIRGULA (Moniez 1887) Kudo 1921

[Fig. 440]

1887	*Nosema virgula*	Moniez	1887a : 1313
1895	*Glugea virgula*	Pfeiffer	1895 : 64
1921	*Thelohania virgula*	Kudo	1921b : 141

Habitat: Fat bodies of *Cyclops* spp.

Locality: France (Lille) and Germany (Weimar).

Vegetative form: Moniez: Sporogenous masses (pansporoblasts) are 30μ by 20μ.

Pfeiffer: The spores are arranged in a stellate form.

Spore: Moniez: Pyriform, one end sharply pointed, the other rounded. Anterior end is often bent to one side. A large vacuole at the rounded end. 8μ by 3μ.

Pfeiffer: Elongated pyriform, one end pointed, the other rounded (Fig. 440). At the latter there is a vacuole. Length 8μ, breadth 5μ.

Remarks: On the basis of Pfeiffer's figure, Kudo (1921) placed the species provisionally in this genus.

THELOHANIA OCTOSPORA Henneguy 1892

[Figs. 441-443]

1892	*Thelohania octospora*	Henneguy and Thélohan	1892a : 621-632
1894	*Thelohania octospora*	Gurley	1894 : 197-201
1895	*Thelohania octospora*	Thélohan	1895 : 361
1920	*Thelohania octospora*	Goodrich	1920 : 17-19

Habitat: The muscles of *Palaemon rectirostris* and *P. serratus*. Henneguy and Thélohan noted that the microsporidian appeared from March to April, that it occurred abundantly in July and August decreasing towards September and October, and that it entirely disappeared after November 15. The parasite invades the muscle fibers and occurred more frequently in the first host (extremely frequent at Le Croisic) than in the second. Other organs of the host such as the alimentary canal, nervous system, glands, reproductive organs remained free from the infection. Infected animals are easily recognized by the chalky opacity which is limited to the muscles affected. When heavily infected the entire body becomes white except the regions of the heart, stomach, some part of the pincers, antennae and abdominal segments which remain transparent. These exceptions constitute the only difference between this condition and the opacity produced by heat or alcohol. The affected muscle fibrillae do not usually show any alternation. Sometimes the elasticity disappears, rupture resulting. The muscle striae, however, remain exceedingly clear no degeneration being observed. The nuclei of the infected muscle fiber are more numerous and smaller in size than normal. The muscular activity is considerably diminished. The animal when more or less heavily infected, loses its active movements to a considerable extent. Among the infected hosts, no egg bearing females were observed, and the condition may probably be a case of parasitic castration. Infected individuals do not survive long, all succumbing by the end of autumn, none being found during the winter. Affected animals are usually found in small shallow ditches where the water is rarely renewed and consequently gains a high temperature. These are probably the conditions favorable to the development of the microsporidian.

Artificial infection experiments failed when tried *per os* on the captive host animals. Henneguy inclined to think that the infection does not take place through the digestive tract in view of the fact that the lesions are found at first at places remote from the alimentary canal. Goodrich found an infected prawn, *Palaemon* (*Leander*) *serratus* in an aquarium tank at Plymouth in January. The prawn with opaque white muscles was in a large tank with several lobsters and many other prawns and shrimps and although nearly all its muscles were affected, it managed to preserve a certain amount of agility. No other prawn from the aquarium could be found to have any trace of infection either in its muscles or blood.

Locality: France (Le Croisic, Concarneau, Roscoff) and England (Plymouth).

Vegetative form: Henneguy and Thélohan: Vesicles, the sporonts, are rounded, 10μ in diameter, and covered with a uniformly thin and transparent membrane. Each pansporoblast forms eight spores which fill a portion of the sporont cavity and are dispersed without order.

Goodrich: The sporocysts (sporonts) are about 8μ in diameter.

Spore: Henneguy and Thélohan: Pyriform; highly refringent (Fig. 441). The polar capsule is present (Fig. 442). The filament is extruded under the action of ether. Length 3 to 4μ, length of polar filament 40 to 50μ.

Thélohan adds that the spore membrane is nonstriated.

Goodrich: Fresh spores are 3μ long, although some are 5 to 6μ long ("macrospores"). Each spore possesses three long tails, 20μ long, which are flattened out proximally but tapering to very fine ends. These tails show up more clearly when dilute iodine is run into the preparation and occasionally the polar filament is shot out from the opposite end (Fig. 443). The polar filament is of uniform thickness and measures 30 to 40μ in length. These tails are hardly visible in preparations stained with Heidenhain or Giemsa and mounted in Canada balsam.

THELOHANIA CONTEJEANI Henneguy 1892
[Figs. 444, 445]

1892	*Thelohania contejeani*	Henneguy and Thélohan	1892a : 637-638
1894	*Thelohania contejeani*	Gurley	1894 : 196-197
1895	*Thelohania contejeani*	Thélohan	1895 : 362

Habitat: The muscles of *Astacus fluviatilis.*

Henneguy and Thélohan note that the infected muscles were white and opaque in the fresh state which could be recognized on the ventral surface of the abdomen. A noticeable diminution of muscular activity was clearly established with the myograph of Contejean. The infection raged with intensity among the crustaceans in the locality for several years. The muscular fibrillae were separated by parasitic masses. They appeared in cross sections as numerous deeply stained punctules and in longitudinal sections as irregular chains separating the fibrillae. The infected fibrillae preserved their normal appearances, the striae being perfectly distinct.

Locality: France (Pontarlier, Department of Doubs, in the vicinity of Lyon).

Vegetative form: Henneguy and Thélohan: Small protoplasmic spheres containing variable number of nuclei are evidently younger phases of the parasite. The complete changes could not be studied. The number of spores surrounded by a common membrane were eight (Fig. 444).

Thélohan: The sporont is spherical and measures about 8μ in diameter.

Spore: Henneguy and Thélohan: Ovoidal (Fig. 445). Size and appearance are similar to that of *T. octospora*. A clear vacuole at the round end. The polar capsule with the filament could not be observed since the material had been preserved. Length 2 to 3μ.

THELOHANIA MACROCYSTIS Gurley 1893

[Figs. 446-448]

1891		Garbini	1891 : 151, 152
1893	*Thelohania macrocystis*	Gurley	1893 : 410
1894	*Thelohania macrocystis*	Gurley	1894 : 205
1895	*Thelohania macrocystis*	Thélohan	1895 : 362

Habitat: The muscle of *Palaemonectes varians*. Artificial infection by inoculation failed (Garbini).

Locality: Italy (Mincio near Verona).

Vegetative form: Pansporoblast elongated fusiform, containing eight spores (Figs. 446, 447).

Spore: Pyriform (Fig. 448). Spore membrane stained only in a 5 per cent. eosin solution when boiled; spores easily stained by Gram's method. Dimensions not given.

Remarks: A doubtful form. According to Gurley, the species is provisionally listed here.

THELOHANIA MÜLLERI (Pfeiffer 1895) Stempell 1902

[Figs. 449-460; 751]

1895	*Glugea mülleri*	Pfeiffer	1895 : 21, 53, 175-182
1901	*Plistophora mülleri*	Stempell	1901 : 157-158
1902	*Thelohania mülleri*	Stempell	1902 : 235-272
1917	*Thelohania mülleri*	Léger and Hesse	1917 : 12-14

Habitat: The muscles of *Gammarus pulex*.

Pfeiffer noted different seats of infection according to the difference in the locality: one or two to three large masses of the microsporidian in the dorsal muscles of the crustaceans were noted in material collected from Papierbach, numerous small masses in the extremities of the host were seen in those caught in the Ilm near Tiefurt, and many scattered small masses were observed in those from Possenbach. The infection was noted throughout the year. Heavily infected animals died in the aquaria more rapidly than normal.

Stempell observed that 18 per cent. of the host animals examined by him were infected. The infected host showed white opaque coloring. The parasite invades the muscular tissue only, others being free from the infection. Younger animals are more heavily attacked than the older ones; the entire muscle when infected shows a whitish opaque appearance. Both the body muscle and muscles of appendages are attacked. Even in case of heavy infection, the host did not show any pathological effect. More or less heavily infected animals could live for three months in a glass dish. It is, however, conceivable that the infected animals die sooner or later. The infection of a new host takes place through alimentary canal. At the end of the second and third days of infection, empty spore membranes were found among apparently unchanged spores. The former appeared to have a small opening at one end. After being in the host gut for 48 hours the spores show the sporoplasm which has shifted its place into one end and contains four nuclei. On the other hand the spores which were kept in stagnant water for three months have only two nuclei in the sporoplasm. Stempell thinks that the nuclear division in this case is a maturation process prior to the germination of the sporoplasm. He noticed a small, 2μ or smaller, uninucleated body in the intestine of the experimentally infected host.

Léger and Hesse studied the microsporidian parasites of Gammarus and stated that there are two different species of *Thelohania* (*mülleri* and *giraudi*) in *Gammarus pulex* which were confused as one and the same species by Pfeiffer and Stempell. *Thelohania mülleri* was observed in hosts living in rapidly running waters. The parasite invades the body musculature, from the posterior part of the head to the tail including the muscles of the legs (Fig. 751). To the naked eye, the body appears to be striped with yellowish white masses which are infected muscular bundles.

Locality: Germany (Weimar, Bierbach, etc; November, December, March) and France (Dauphine).

Vegetative form: Pfeiffer: small spherical pale-looking bodies 8 to 10μ in diameter. Each contained 8, 16, 24 or 32 spores.

Stempell: "Meronts" (i.e. schizonts) highly variable in size and form, due to division. The ordinary meronts are spherical, but assume various forms when in division. The meront (Fig. 449) is from 2 to 6μ in diameter. Its cytoplasm seems to be of a fine reticular structure and takes stain more deeply than that of the sporont. Amoeboid movements were not observed, though it is probable that the schizont can move. The resting stage shows a pellicula-like layer, ectoplasm and endoplasm (Fig. 450). Each schizont contains a small nucleus well stained with Delafield. The chromatic granule is surrounded by a clear zone. The meronts divide by binary fission or by budding. The nuclear division is typical amitosis. Frequently, nuclear division proceeds without cytoplasmic constriction, thus forming sausage-form meronts with many nuclei or chain forms (up to eight individuals; Figs. 449, 452). Sometimes abnormal schizonts, of 16 to 18μ in diameter, contain 12 or more spores. Whether the meronts can actually invade other muscle bundles or not, could not be determined. By growth, each schizont becomes a sporont.

The sporonts are pale-looking spherical bodies and are 8 to 10μ in diameter. The cytoplasm is less dense and stains less deeply than that of the meronts. The ectoplasm is hardly visible. The nucleus resembles that of the schizont, assuming often, however, a horse-shoe shape. It divides amitotically and repeatedly, forming eight daughter cells (Figs. 453-456). Each of these sporoblasts becomes elongated, takes a pyriform shape and becomes a spore.

Léger and Hesse: The microsporidian infiltrates all the muscles. Under a magnifier, the infected muscle is seen to be replaced by whitish fusiform masses which are composed of sporonts and spores. Fully formed sporonts are spherical bodies and are 7 to 8μ in diameter (Fig. 459). The pansporoblast membrane is hyaline, fragile and hard to stain.

Spore: Pfeiffer: Pyriform. A vacuole at one end. Polar capsule invisible. The filament is extruded under the action of acetic or nitric acid. Length 3 to 4μ, length of the filament 15μ.

Stempell: Pyriform (Fig. 457). A small vacuole at the anterior end and a large one at the rounded posterior end. The latter vacuole shows often artefacts due to the action of reagents. Minute structure could not be seen in the fresh state. Spores mounted in Canada balsam do not show the membrane so that they appear much smaller than fresh spores. Whether the smaller vacuole is the polar capsule or not cannot be determined; it seems probable however because of the fact that the filament was extruded from the narrow end. Mechanical pressure or iodine alcohol brought out the filament (Fig. 458). The nucleus in the sporoplasm divides into two by amitosis. Abnormal spores reach 6μ up to 12μ in length. Fresh spores, length 4 to 5μ, breadth 2μ, length of polar filament 22 to 24μ.

Léger and Hesse: Typically ovoidal (Fig. 460). The spores in the sporonts presented various forms, mostly slightly kidney-shaped. Length about 5μ.

Remarks: Another microsporidian was described by Debaisieux (1919a) as *Glugea mülleri* which possesses entirely different trophic phases from the present species, although both Stempell and Debaisieux refer the forms they studied to the species described by Pfeiffer as *Glugea mülleri*.

THELOHANIA VARIANS (Léger 1897) Debaisieux 1919

[Figs. 461-489, 771, 775]

1897	*Glugea varians*	Léger	1897 : 260-262
1897	*Glugea varians*	Léger and Hagenmuller	1897 : 552-555
1919	*Thelohania varians*	Debaisieux	1919 : 47-62, 66-69

Habitat: The general body cavity of the larvae of *Simulium ornatum* and in the adipose tissue of the larvae of *S. reptans.*

Léger noticed the parasite in the body cavity of the first host in irregularly contoured, whitish opaque sacs. The number of cysts found in one host, was one (some reaching 0.5 mm. in diameter), two, three or rarely more, and these filled the host's body cavity. In the case of heavy infections the cysts by excessive growth pushed out the integument and produced more or less spherical hernia in the host abdomen. In most cases, however, the cysts as they grew simply pressed aside the organs in the cavity. The muscle was not attacked so that the larvae even when heavily infected, showed active movements. The fat body of the host decreased in quantity, which indicated that the parasite grew at the expense of the host tissue. A young nonsporulating form was once seen on the surface of the alimentary canal in form of a hernia which showed that the migration of the vegetative form from the alimentary canal to the coelom probably took place at an earlier stage of development. Artificial infection experiments were unsuccessful.

Debaisieux remarked that some of the larvae examined were found to be infected. The infected larva showed an opaque whitish coloration at the posterior portion of the body which was more or less distended as a result of the infection. Some parasitic masses appeared as herniae on the surface of the body. The microsporidian develops at the expense of the fat body. One or more irregular parasitic masses were found in the general body cavity at the posterior portion of the host body. The host nuclei were hypertrophied (Figs. 771, 775).

Locality: France and Belgium (in the vicinity of Louvain; end of winter).

Vegetative form: Léger: The cyst is covered by a thin membrane. The spores are of two kinds. The pansporoblasts contained one, two, four or eight nuclei which corresponded to different stages of development.

Debaisieux: The youngest schizonts are generally binucleate, the nuclear division taking place either in the spore or immediately after the liberation of the sporoplasm. The schizonts' nuclei multiply by amitosis and the daughter nuclei remain in pairs for a long time (Figs. 462 to 464). Schizogony takes place in two different methods which are binary fission and multiple division (Figs. 465 to 467, 469 to 473). The final products of schizogony are binucleated "autogamous diplocarya", the two nuclei of which were formed by the division of the parent nuclei (Figs. 468, 474).

The autogamous diplocaryon is an irregular but generally elongated body. Its protoplasm is uniform in structure and less distinctly contoured than that of the schizont. The nuclei are voluminous with a more or less stainable network and each possesses an irregular chromatic mass. They extrude small granules into the cytoplasm. This process occurs simultaneously and symmetrically in the two nuclei and the granules finally disappear completely (Figs. 474, 476). The large chromatic granule in the nucleus breaks up into smaller masses, and these become scattered over the chromatic network which produces long filaments disposed more or less radially in each nucleus. The membrane separating the two nuclei disappears and the chromatic filaments of the two become united (Fig. 477). The zygote thus formed is the sporont (Fig. 478). Its nucleus becomes elongated without passing through a resting period and condensed at the extremities, the division being amitosis (Figs. 479, 482). The nuclei divide twice more, passing through a resting stage after each division. Finally the sporont contains eight nuclei (Fig. 485). In its cytoplasm, there are to be seen some chromatic granules which develop enormously during the first nuclear division (Figs. 479-482). These granules play probably a rôle in the formation of the spore membrane. The transformation of a sporoblast into a spore which is similar to that in *Plistophora longifilis* studied by Schuberg, is hard to follow. One sees a large nucleus with a reticular structure, lying at one end of the cytoplasm, while an elongated vacuole appears at the other end (Fig. 486). The membrane which appears later, seems to be a continuous piece and there is no evidence that it consists of two valve-cells. The spore membrane is probably differentiated from the peripheral cytoplasm of the sporoblast together with the substance which fills the sporont. In the meantime the sporoplasm assumes a pear-shape and a large nucleus is located at the round end, while a vacuole becomes prominent at the other end where metachromatic granules are seen (Figs. 487, 488). These granules are often scattered throughout the spore and stain red with Giemsa or Romanowsky, while the nucleus takes a blue color. One or more vacuoles are found at the broad end. A polar capsule was not observed.

Spore: Léger: Ovoidal. A vacuole at the rounded end. Refringent. The filament was extruded from the pointed tip of the spore under the influence of iodine water. Dimorphism in spores (macrospores and microspores). They occur either in the same or in different cysts. The former are found lying in spherical masses in variable numbers, while the latter, eight in group, are seen surrounded by a thin membrane. Macrospore about 8μ long and microspore about 4 to 5μ long.

Debaisieux: Form pyriform or oval (Fig. 489). A single nucleus usually located in the center. Vacuoles are irregularly situated. No polar capsule is noted. The filament is coiled directly under the shell. Metachromatic granules occur in variable number and position. Certain spores develop new infections in the same host by liberating their amoebulae. The filament was extruded under the influence of very dilute hydrochloric acid. Size greatly variable. Fixed specimens measured after Debaisieux's figures: Length 6.5 to 8μ, breadth about 4.5 to 5.5μ.

Remarks: Debaisieux unfortunately did not compare his form with Léger's species thoroughly, not even giving the dimensions of various stages.

THELOHANIA PINGUIS Hesse 1903

1903	*Thelohania pinguis*	Hesse	1903a : 418
1904	*Thelohania pinguis*	Hesse	1904 : 3-4

Habitat: The fat body of the larvae of *Tanypus varius* (Diptera). Two out of one thousand host larvae examined were found to be infected by the microsporidian. The microsporidian produces voluminous tumors that fill the body cavity.

Locality: France.

Vegetative form: The pansporoblasts are spherical, 6 to 6.5μ in diameter or ellipsoidal, 7μ by 4μ. Each contains eight spores.

Spore: Generally ovoidal, sometimes pyriform. Glycerine caused filament extrusion in fresh spores. Length 3 to 3.5μ, greatest breadth 2μ, length of polar filament 20μ.

THELOHANIA JANUS Hesse 1903

1903	*Thelohania janus*	Hesse	1903a : 419
1904	*Thelohania janus*	Hesse	1904 : 4

Habitat: Fat body of the larvae of *Limnophilus rhombicus* (Diptera). Of very rare occurrence. The microsporidian formed voluminous clusters in the thorax and the posterior portion of the abdomen.

Locality: France (in the vicinity of Grenoble).

Vegetative form: The pansporoblast with macrospores is spherical, 5μ in diameter, or ellipsoidal, 4.5μ by 5.5 to 6μ. It contains four macrospores. The pansporoblast with microspores is always spherical, about 5.5μ in diameter. It contains eight spores.

Spore: Macrospore is reniform and measures 6μ long by 2μ broad. Microspore is ovoidal but not curved and measures 3μ long by 2μ broad. The filament of the microspore measure 24 to 25μ in length. When the spores are treated with iodine water, the microspore extrudes its polar filament, while the macrospore does not.

THELOHANIA MAENADIS Pérez 1904

[Figs. 490-498]

1904	*Thelohania maenadis*	Pérez	1904 : 214-215

1905	*Thelohania maenadis*	Pérez	1905 : 2-11, 18-22 1905b : 148-149
1906	*Thelohania maenadis*	Pérez	1906 : 1091-1092

Habitat: The muscle and ovary (in one case) of *Carcinus maenas* (Crustacea). The pansporoblasts are found in large numbers and form fusiform dense masses which are embedded in the striated muscle fibers. In certain regions, these masses replace the muscle fibers entirely (Fig. 490), in which case the nuclei of the muscle cells are found among the sporonts. The infected muscle shows an opaque white coloration. The host blood becomes milky white and is less coagulable while the microsporidian is undergoing active schizogony. Both infected male and female reproductive organs did not show any particular histological changes as in *Nosema pulvis*.

Locality: France (Arcachon).

Vegetative form: The youngest meronts are spherical, about 5μ in diameter, with a membraneless nucleus which shows usually four, but sometimes three or five, cuneiform chromatic granules (Fig. 491). The meronts divide by binary fission into two, sometimes by multiple division into three or four daughter meronts (Figs. 492 to 494). The nucleus of the daughter schizonts assumes a homogeneous and compact form. These chromatic granules distinguish themselves as refringent particles in the fresh state. Larger schizonts, 8 to 9μ in diameter, are also found. These large forms undergo either binary (Fig. 495) or multiple fission. The chromatic substances are separated into eight groups, each assuming a Y-shape. Passing through various stages, the nucleus divides. The nuclear division is a typical mitosis with complete absence of the achromatic figure. The size of the dividing form is 8 to 9μ, those in biscuit shape 10 to 11μ, some forms in multiple schizogony reach 15, 20 or 25μ in diameter.

The meront finally becomes a sporont (Fig. 496). The chromatic substance breaks up into numerous and much smaller granules, while the body reaches a size of 12 to 13μ in diameter. The cytoplasm contains refringent enclosures which are stained deeply with osmic acid. The chromatin is nebulous and is now almost invisible in the fresh state. In developing into a pansporoblast, the nebulous nuclear substances of the sporont break up into ten, sometimes into nine groups (Fig. 496). Eight of these form eight spores, the other two (or one) remain in the pansporoblast as residual nuclei, appearing as compact masses (Fig. 497). The spores are arranged in the pansporoblast without any order.

Spore: In fresh state, a clear vacuole is seen at the more rounded end (Fig. 498). The polar capsule is not made visible by addition of reagents. Various reagents failed to cause filament extrusion. Length 5μ, breadth 4μ.

THELOHANIA LEGERI Hesse 1904
[Figs. 499-507, 694-727, 764; Textfig. H]

1904	*Thelohania legeri*	Hesse	1904a : 570-571 1904b : 571-572
1921	*Thelohania illinoisensis*	Kudo	1921a : 167-171, 177
1922	*Thelohania illinoisensis*	Kudo	1922 : 74-75
1924	*Thelohania legeri*	Kudo	1924 : 147-162
1924	*Thelohania legeri*	Kudo	1924a : (in press)

Habitat: Fat bodies of the larvae of *Anopheles maculipennis* and *A. bifurcatus* (Hesse); of the larvae of *A. punctipennis*, *A. quadrimaculatus* and *A. crucians* and also of adult (both male and female) *A. quadrimaculatus* (Kudo).

Hesse found two infected larval *A. maculipennis* out of 40 he examined (1904) and further according to his preparations some infected larvae of *A. bifurcatus* (1918, 1919). The digestive tract was not infected. Although no adults were examined he concluded that apparently infection occurs among the adults since the infected larvae did not seem to suffer from the infection.

Kudo found in different localities of the United States several cases of infection of anopheline mosquitoes by the microsporidian; in 1923 he examined 1250 host larvae, mainly *A. quadrimaculatus* and *A. crucians* in the vicinity of Leesburg, Georgia, and found 54 infected. Kudo (1924a) found the microsporidian in four adult *A. quadrimaculatus* from the same locality. In the case of heavy infection, the host larvae showed typical opacity and inactivity of the body. Due to enormous growth of the parasite in the fat body, the body often becomes deformed and presents a characteristic appearance, called "dough-belly" (Barber). Such larvae succumb to death very easily as compared with the normal larvae. But there is no doubt that in the case of light or moderate infection, infected larvae would metmorphose into pupae and further to adults. Kudo succeeded in causing artificial infection of normal larvae by feeding the microsporidian *per os*. He attached important economic significance to the parasite.

Locality: France and the United States (Illinois, New York, Georgia, Alabama).

Vegetative form: Hesse: The meronts are rounded bodies, measure 3 to 4μ in diameter and possess deeply staining cytoplasm and a nucleus formed by a group of chromatic granules, surrounded by a clear zone (Fig. 499). Schizogony is by binary fission as in *T. mülleri* worked out by Stempell, the nuclear division being direct. Chain forms are often encountered, up to three individuals. The nuclear division is not always followed by immediate constriction of the cytoplasmic body. The meront ultimately becomes a sporont (Fig. 500). The sporonts are oval without membrane, 9 to 10μ in length and 4 to 6μ in breadth. The cytoplasm is less stainable than that of the schizont. The nucleus is large and with a distinct nuclear membrane; fine chromatic granules are scattered on the network and a complex karyosome is formed by four chromatic granules. The nuclear division is a sort of mitosis, with a spireme stage. Division continues successively, producing eight nuclei arranged in a rosette form (Figs. 501-504).

Kudo (1924): The youngest stage is an oval or rounded body with compact chromatic granules (Fig. 694). The latter may be composed of one large and four or five minute granules scattered in the cytoplasm or five or six equally large grains clustered to form a mass. These compact grains transform sooner or later into a vesicular nucleus (Fig. 695). As the cytoplasmic body grows the nucleus undergoes division. In this the entire achromatic substance becomes an irregularly coiled thread with attached chromatin granules. The body is elongated in the direction of the nuclear division, while the thread becomes separated into two groups or parts which at first are connected with each other by a rather thick strand, but which later become independent (Fig. 696). The schizont now divides into two daughter schizonts. Binary fission is repeated while there is a large space left in the host cell which is usually the case at the beginning of the infection.

Some of the schizonts which contain two daughter nuclei, remain without cytoplasmic constriction and grow in size. In the meantime, each of the two nuclei undergoes division (Fig. 697). Division takes place simultaneously so that when it is completed, a stage with four nuclei is produced, the two daughter nuclei being in pairs in the opposite portions. These stages are found chiefly in the peripheral portion of the host tissue the central portion of which is occupied by the stages of sporogony. This tetranucleated body divides into two, each containing two nuclei (Figs. 698, 699). These binucleated schizonts are in reality what one may term sporont mother cells, since they seem to undergo a peculiar division once and form binucleate cells. The nuclei lose their vesicular appearance and assume a compact form in which one may occasionally distinguish chromatic and achromatic portions (Figs. 698, 699). There appear two deeply staining granules at the opposing extremities (Fig. 699). Each nucleus divides into two parts which remain connected with a single strand even after they are widely separated. The divisions of the two nuclei are always simultaneous so that one sees stages such as shown in figures 700 to 702. The body now divides into two parts, each half possessing two nuclei, derived from two nuclei and not two daughter nuclei (Fig. 703). These nuclei are characterized by the possession of a conspicuous oval chromatin grain usually located eccentrically in the achromatic network. These two nuclei grow large by a considerable increase in the nuclear sap and come to lie side by side (Fig. 704). In the meantime, the chromatin substances become strikingly conspicuous and spread over the achromatic network, and the wall between the two nuclei disappears. The nuclear substances fuse into one mass, while a large number of chromatin granules of variable size appears in the cytoplasm (Fig. 705). The granules are apparently thrown out by the nuclei prior to and during the nuclear fusion. The resulting form has a large nucleus with chromatin grains attached to the achromatin network and to the nuclear membrane and cytoplasm in which chromatin granules of variable size and number are imbedded. This is the sporont (Fig. 706).

The nucleus of the sporont, after passing through a short resting stage, undergoes a division. The chromatin forms a spireme which at first is rather fine and closely convoluted (Fig. 707), but later thickens and shortens (Fig. 708). At this latter stage one can see clearly that the thread is single. The nuclear membrane becomes less conspicuous and the entire nucleus assumes an elongated form, the axis coinciding with the general direction of the spireme. The latter breaks up to form rounded or oblong chromatic granules which may be called chromosomes (Figs. 709 to 712). Whether these chromosomes are transversely broken threads or not cannot be determined, owing to the minuteness of the object. The achromatic network becomes stretched in the direction of the nuclear division and forms a sort of a spindle (Figs. 710, 712), at the equatorial plane of which the chromosomes become located. The number of chromosomes seemed to vary: in one stage, six were seen, in another over ten were counted, but in the anaphase, fairly regularly eight chromosomes were seen in two groups of four moving toward the opposite ends (Fig. 711). The two groups of the chromosomes finally reach the opposite ends and the two daughter nuclei are constructed (Fig. 713). The nuclei thus formed have a large chromatin mass, usually located eccentrically, from which radiating chromatic threads reach the nuclear membrane. To the latter are attached the chromatin granules. Each of the nuclei soon divides twice more (Figs. 714 to 722). Finally the sporont contains eight compact nuclei, each of which becomes surrounded by an island of cytoplasm and transforms into a sporoblast. The sporulation seems to follow the changes observed in *Stempellia magna*.

Spore: Hesse: Ovoidal, with nearly equal extremities (Fig. 505). After fixation with acetic sublimate, the anterior end of the spore becomes deformed and flattened. Spore membrane thick and distinct. The polar filament is easily extruded under the action of iodine water. When the spore is stained with Heidenhain, it shows a large central mass that represents the polar capsule and the sporoplasm (Fig. 506); with Romanowsky four closely located primitive nuclei become differentiated in the central mass (Fig. 507). Macrospore 12μ long by 5μ broad. Microspore 6 to 8μ long by 3 to 4μ broad, length of polar filament about 50μ.

Kudo: Oval, with equally rounded extremities (Figs. 725, 726). Less refractive than *Nosema bombycis* or *N. apis*. No dimorphism. In the fresh state, the spore membrane is clearly seen separated from its contents. The contents of the spore are very peculiar in shape; one end is narrow and truncate, the other regularly rounded along the inner surface of the membrane. The contents are either uniformly granular or with a vacuole, rounded triangular in form. Fresh spores measure 4.75 to 6μ long by 3 to 4μ broad.

When fixed the spore membrane becomes truncate at the end in which a vacuole is noted (Fig. 727). The sporoplasm is located near one end, while the polar capsule occupies a large space in the spore. The filament was extruded under the action of mechanical pressure and is 60 to 97μ long.

THELOHANIA CEPEDEI Hesse 1905

1905 *Thelohania cepedei* Hesse 1905a : 919

Habitat: Malpighian tubules of *Omophlus brevicollis* (Coleoptera). The spores are found in the lumen and the developing stages are located in the epithelium of the tubules.

Locality: France.

Vegetative form: The pansporoblasts are spherical and 7 to 9μ in diameter. Each pansporoblast contains eight spores, though some contain only four spores in which case the spores are twice as large as the normal spores.

Spore: Form and dimensions vary greatly. Oval, ellipsoidal or arch-shaped. A vacuole at the posterior end is well visible in most spores. The vacuole often fills up the intrasporal space. The polar capsule is recognizable without any treatment and the filament is extruded under the influence of iodine water. Length 3 to 6μ, breadth 2 to 2.5μ, polar filament is 20 to 25μ long.

THELOHANIA sp. Mercier 1906

1906 *Thelohania* sp. Mercier 1906 : 90-91

Habitat: The muscles of the body and heart of *Talitrus* (?) sp.

Locality: France (Roscoff).

Vegetative form: The meront is a small spherical body and contains a nucleus composed of four chromatic grains. The schizogony observed by Pérez in *T. maenadis* was not noticed. The meronts penetrate through the muscle fibers where they form long lines. The nucleus becomes vesicular and shows a membrane and a large central nucleolus. Fusion of two such uninucleated bodies takes place and produces a binucleated body. The nucleoli break up into fragments which lie at the periphery under the membrane. When the nuclear membrane disappears, the chromatic granules move out to the peripheral part in pairs. These chromatic grains fuse two by two and become grouped into 9 or 10 masses. Eight of these constitute small distinct nuclei, the remaining one or two forming residual nuclei. The sporont now becomes a pansporoblast. Each of the eight nuclei develops into a spore by condensing an island of cytoplasm around it. The ultimate stages of the pansporoblasts are found in the muscle fibers of the heart. Thus the species stands in contrast with *T. maenadis* which, according to Pérez, does not invade the heart.

Spore: Dimensions and other observations on the structure are not given except that Mercier could not ascertain the presence of the coiled filament in the spore and filament extrusion was unsuccessful.

THELOHANIA BRASILIENSIS Kudo 1924

[Fig. 508]

1908 *Nosema corethrae* Lutz and Splendore 1908 : 315

Habitat: In the larva of *Corethra* (*Savomyia*) sp. The infected host larvae showed white spots to the naked eye. These spots are groups of cysts, each of which contains eight spores.

Locality: Brazil.

Spore: Pyriform or curved cylindrical form (Fig. 508). A round vacuole at the slightly broader extremity. Length 5.5 to 7.5μ, breadth 1.5 to 2μ.

Remarks: The octosporous character mentioned by the authors marks the species as one of Thelohania. As the observation is incomplete, it is separated from *T. corethrae* Schuberg et Rodriguez, doubtless a closely allied form. It is unfortunate that the latter two authors failed to mention the dimensions of the species which they found in Berlin.

THELOHANIA CHAETOGASTRIS Schröder 1909

[Figs. 509-518, 777-779]

1909 *Thelohania chaetogastris* Schröder 1909 : 119-133

Habitat: The connective tissue and muscle cells of *Chaetogaster diaphanus*. The animals caught in autumn and kept in the laboratory, showed more or less heavy infections in the winter. The worms which are ordinarily perfectly transparent had an opaque white appearance in the posterior region of the body. Isolated spores were found to be floating in the body cavity. Eight infected host animals were examined; two apparently normal at the time of collection developed a similar infection while in the dish for eight days. The nuclei of the host cell become vesicular and the chromatic granules become attached to the nuclear membrane. Nais and Stylaria showed upon examination that they were free from the infection of the microsporidian.

Locality: Germany (near Heidelberg).

Vegetative form: The youngest schizonts, less than 10μ in diameter, are found in connective tissue cells and rarely in muscle cells. Their irregular forms suggest amoeboid movements. The cytoplasm is dense, stains deeply and contains numerous nuclei about 1μ in diameter, which are structureless. The schizonts (Fig. 511) divide rapidly and repeatedly without a complete separation into daughter individuals, thus forming a rosary. Each daughter schizont has usually two nuclei, rarely one or three. This stage is followed by an elongation of the body which becomes dumbbell shaped and divides into two (Fig. 511). The final stages of schizogony are uninucleated spherical bodies of about 3μ in diameter (Fig. 512). Whether this form further divides by binary fission or grows up into a multinucleated stage, is not clear. The protoplasmic bridge between the dividing schizonts is not the ectoplasm, but only the less dense cytoplasm.

The sporonts are found exclusively in the cysts, while the schizonts occur freely in the cytoplasm of the host cells. Cysts often contain not only the various stages of spore formation, but also the schizonts. The advanced stages are in the central portion and the young stages at the periphery in the cyst. The latter reaches 100μ in diameter, occupying numerous host cells. The young stage in sporogony is a uninucleated or binucleated spherical protoplasmic body of about 3μ in diameter (Fig. 513). Its cytoplasm is less dense, reticulated and stains less deeply than that of the schizont. Whether the binucleated schizonts are formed by the division of the nucleus in the uninucleated form or by copulation of two such forms could not be determined. The binucleated form is followed by one with four nuclei which are arranged in pairs at the extremities of the dumbbell-shaped body (Figs. 513, 514). Further division of the body results in forming a cross, each portion with a single nucleus (Figs. 514, 515). By another division of these four nuclei, there appears a stage containing eight sporoblasts which are connected with one another by a central residual mass (Figs. 515, 516). The pansporoblast membrane is very delicate and can be made out only in sections. The nucleus is often surrounded by a clear space and is at the distal end. In the middle of the sporoblast spherical or oval vacuole and sometimes also a slightly deeply staining oval body are noticed. The nuclear division is probably mitotic. The next stage was a young spore. In this the cytoplasm becomes condensed at the middle portion, forming two vacuoles at the extremities. The central part is occupied by the oval polar capsule, its opening being drawn out obliquely to one end of the spore. The number of nuclei is five in all; two in the sporoplasm, one in the polar capsule, and two in the valves of the spore membrane.

Spore: Ellipsoidal and circular in cross-section (Figs. 517, 518). It is somewhat smaller than the sporoblast. The polar capsule is fairly well noticeable. No attempts for the extrusion of the filament were made. The nuclei of the spore membrane disappear when the latter is formed. The sporoplasm usually contains one or two nuclei. There probably occurs a nuclear union in the spore as in Myxosporidia. Macrospores, in a few cases, measure 6μ long by 4μ broad. Microspores, occurring in large numbers, measure 4μ long by 3μ wide.

THELOHANIA GIRAUDI Léger 1909
[Fig. 519, 752]

1909	*Thelohania giraudi*	Léger	1909 : 210
1917	*Thelohania giraudi*	Léger and Hesse	1917 : 12-15

Habitat: The muscle of the posterior dorsal or latero-dorsal portion of the body of *Gammarus pulex* (Fig. 752). The infected animals are encountered in still water, while those attacked by *T. mülleri* are rare and found in rapid running water. The microsporidian produces in the host body one tubular mass (often two or three, rarely more) which is clay-white and easily distinguished from the greyish yellow body. The tubular masses reach from 2 to 3 mm. in length and can easily be isolated from normal muscular tissue by means of a needle.

Locality: France (Dauphine).

Vegetative form: The tubular masses contain at the end of multiplication innumerable numbers of sporonts with spores. The sporonts vary in form even in one and the same host individual. Small spherical sporonts contain eight spores (Fig. 519), while larger forms 16, 32 or more spores. On the other hand a very small sporont with four spores was encountered. The sporont with numerous spores is ovoidal, wallet-form or polyhedral with rounded angles. The octosporous sporonts about 9.5μ in diameter are not so numerous as those with 16 or 32 spores. The sporont membrane is thick, resistant, well stained and rigid, a structure not seen in *T. mülleri*.

Spore: Ovoidal (Fig. 519). Slightly more elongated than the spore of *T. mülleri*. Kidney-shaped spores do not occur. Length 5.5μ, length of the polar filament 60μ.

Remarks: Léger and Hesse think that the species was confused with *T. mülleri* by Pfeiffer and Stempell. Compare also *Glugea mülleri*.

THELOHANIA OVICOLA (Auerbach 1910) Kudo
[Figs. 520-524]

1910	*Plistophora ovicola*	Auerbach	1910 : 774-776

Habitat: The egg of *Coregonus exiguus bondella*. The infected eggs were easily distinguished from the normal ones by their milky white coloration.

Locality: Switzerland (Lake of Neuchatel).

Vegetative form: The young form observed was a somewhat oval sporont, about 6μ in length and with two dividing nuclei (Fig. 520). In more advanced stages the sporont, 6 to 10μ in diameter, does not show the nuclei distinctly. It is spherical, and is surrounded by a clear membrane. The sporonts with sporoblasts or mature spores are either spherical or somewhat irregular and measure about 10 to 12μ in diameter.

Spore: Oval; often the middle part is constricted (Figs. 521, 524). Size variable. Spore membrane thick. In glycerine, vacuoles are observable at each end (Fig. 522). When stained the sporoplasm shows four small nuclei. Many spores when kept in glycerine extruded their polar filament (Fig. 523). Length 6 to 8μ, breadth 4 to 6μ (some larger), length of polar filament 25 to 30μ.

Remarks: Auerbach's figures and the dimensions of the sporulating sporont and the spores lead this species to be placed in the present genus rather than in Plistophora.

THELOHANIA BRACTEATA (Strickland 1913)
Debaisieux et Gastaldi 1919

[Figs. 525-531]

1904	*Nosema simulii* γ	Lutz and Splendore	1904 : 647
1913	*Glugea bracteata*	Strickland	1913 : 66-71
1919	*Thelohania bracteata*	Debaisieux and Gastaldi.	1919 : 194-196

Habitat: The fat body of the larvae of *Simulium venustum* and *S. ochraceum* (Lutz and Splendore), *S. bracteatum* and *S. hirtipes* (Strickland), and *S. maculata* (Debaisieux and Gastaldi).

Strickland noted about 10 per cent. of the larvae examined were infected. When the infection is at an advanced state, one sees a large irregular white mass, sometimes measuring as much as 2 mm. in length, which naturally causes the distension of the host's abdomen. The fat body of the infected host is usually considerably diminished in quantity. Infected larvae died in captivity much more quickly than the normal ones, death often being due to the skin rupturing. No infected pupa was found. The infected larvae never showed any brown coloring as do the normal ones before pupation.

Lutz and Splendore found the infection only in a small percentage of the larvae they examined. The infected larvae showed very white tumors in the hinder portion of the body. The tumors lie apparently free in the adipose tissue between the intestine and the body wall.

Debaisieux and Gastaldi state that the tumors are mostly irregularly contoured. The different stages intermingle with one another without order and isolated spores were noted in the center. There is no true membrane outside the tumor. The nuclei of the infected host cells become hypertrophied and altered.

Locality: Brazil, the United States (in the vicinity of Boston; autumn) and Belgium (in the vicinity of Louvain; October to March).

Vegetative form: Lutz and Splendore noted the octosporous character.

Strickland: The earliest stage ("Myxosporidium") found was a multinucleated mass of protoplasm measuring 2 to 3 mm. in length, in which no definite ectoplasm and endoplasm could be distinguished. The central, and larger, portion of the mass had already sporulated and contained ripe spores; surrounding this area were all stages of development up to the as yet almost undifferentiated thin layer of protoplasm which still persisted around the edge of certain parts of the mass and represented all that was left of the true meront (?) stage. In stained sections, there is a very definite differentiation between the ripe spores and the early stages of development; the former stain intensively with hematoxylin, whereas the latter assume an orange color. Sporonts (Fig. 526) are typically formed by the concentration of the protoplasm ("myxoplasm")around the numerous nuclei in the mass ("myxosporidium") and this results in the formation of small globular bodies, around which the cytoplasm hardens to form a fine membrane. The sporont is a spherical body, about 5.8μ in diameter. It grows reaching 10μ in diameter and contain eight readily visible rounded bodies, the nuclei (Fig. 527). Normally the sporont membrane persists till the complete spores are formed, when it disappears. The mature sporoblast measures about 3μ in diameter. The protoplasm stained deeply, while its nuclear material could not be differentiated by any stains. As the sporoblast matures, a vacuole appears in the center and travels toward the periphery; the cytoplasm becomes more condensed on the side ot the vacuole. At the same time, the cell assumes a more elliptical form and its superficial layer becomes differentiated into a thick shell. The ripe spores remain attached to one another in aggregates of eight. In the young spore the nucleus occupies the extremity of the cell opposite to that occupied by the vacuole. At first, it is rather diffuse but later becomes more concentrated to form a small round body. The cytoplasm becomes still further condensed till it is drawn away from the wall of the spore around the equatorial region, later being pressed back against the spore membrane. Two nuclei and later four are to be seen in the sporoplasm.

Strickland gives the following assumption as to a part of the life cycle of this as well as the two succeeding species: The spore when taken into the mesenteron of a very young host larva, liberates the amoebula after the extrusion of the filament under the influence of the digestive fluid of the host. As the peritrophic membrane has not been completely formed in the host gut, the amoebula is able to come in contact with the gut epithelium and to work its way into the body cavity through the intercellular space. After the peritrophic membrane is completely formed, the spore or the sporoplasm must pass out through the intestine with the feces without attacking the host. The amoebula, after entering the body cavity, lives freely in the blood plasma, but probably does not have any activity such as denoted by planont-stage in other forms. The amoebula enters an adipose tissue cell and becomes a schizont.

Debaisieux and Gastaldi: The changes are similar to those of *T. fibrata* and *T. multispora*. All the stages however are much smaller than those of *T. fibrata*. The plasmodia are rare. On the other hand advanced diplocarya stages were frequently seen. The sporont is formed by the fusion of two nuclei. Large peripheral chromatin granules are observed in this species. The nuclear divisions of the sporonts are very simple, the daughter nuclei being connected by two chromatic filaments. Eight sporoblasts are formed in each sporont (Fig. 529). Eight spores are ultimately produced from these sporoblasts.

Spore: Lutz and Splendore: Size 3.5μ by 2.5μ. The polar filament is 35μ long.

Strickland: Short elliptical, with somewhat truncated ends. The form and size are extremely uniform, only two large spores measuring 8μ by 5μ being noticed. A small vacuole is seen in fresh spores. Treated with iodine, the spore shows the inner wall of the thick membrane, while in a very few cases (less than one out of every thousand) a rather stout polar filament is extruded (Fig. 528). This may be due to the immaturity of the spores. Length 3μ, breadth 2.5 to 2.7μ; length of the filament 6 times the length of the spore.

Debaisieux and Gastaldi: Regularly subspherical and not affected by reagents, staining intensively. A few macrospores occur (Fig. 531). Fixed spores show two vacuoles, one at each end (Fig. 530). A lenticular protoplasm is located in the middle. In one of the vacuoles, two granules connected with a thread-like structure are present, while in other one turn of the spirally coiled filament which appears short is seen (Fig. 530). Length 3 to 4μ, breadth 2.5 to 3μ.

Remarks: The microsporidian parasites of Simulium larvae seem to belong to more than one species. The synonyms for this and the following two species are given in accordance with Debaisieux and Gastaldi's view

THELOHANIA FIBRATA (Strickland 1913)

Debaisieux et Gastaldi 1919

[Figs. 532-542; 766]

1908	*Nosema simulii* δ	Lutz and Splendore	1908 : 312-313
1913	*Glugea fibrata*	Strickland	1913 : 71-74
1919	*Thelohania fibrata*	Debaisieux and Gastaldi	1919 : 192-194

Habitat: Fat body of the larvae of *Simulium venustum, S. ochraceum* (Lutz and Splendore), *S. bracteatum, S. hirtipes* (Strickland) and ?*S. maculata* (Debaisieux and Gastaldi).

Strickland states about 5 per cent. of the larvae examined were infected. The microsporidian appeared as several large irregular milky white masses which spread through the entire body although they were most voluminous in the swollen apex of the abdomen (Fig. 766).

Debaisieux and Gastaldi state that the tumors which are single or multiple, are irregularly contoured and found among the various organs of the host. Different developmental stages of the microsporidian are found in the tumor, around which there is no particular membrane. The host nuclei enclosed in the tumor are relatively numerous. They are voluminous and contain "chromosomes" which appear as piled dishes. The nuclei of the salivary gland cells thus hypertrophied reach 170μ in diameter.

Locality: Same as the last mentioned species.

Vegetative form: Strickland: In the "myxosporidium" (infected host cell) there are numerous nuclei each of which forms a sporont 5.5μ in diameter (Fig. 533). The sporont grows until it measures about 12μ in diameter when the nucleus divides. The division seems to be of two forms. In one the nucleus becomes ring-shaped and this ring draws out, while in the other the globular chromatic mass simply divides by amitosis to form two hemispherical masses (Fig. 534). Each of the nuclei thus formed divides again. The division was seen only twice and in each case the nuclear matter had evidently assumed a ring-like form before division (Fig. 535). In the next stage, there was evidently a third nuclear division forming eight nuclei. The sporont wall becomes indented between the eight regularly spaced nuclei. This indentation advances until each nucleus is surrounded by an island of cytoplasm (Fig. 536). Each of these eight sporoblasts when free, is a spherical body which measures at first about 5.2μ in diameter, but later increases somewhat till it measures about 6.2μ. The sporulation is apparently similar to that of *T. bracteata* except that in this species the spores are entirely free throughout the whole development.

Debaisieux and Gastaldi: Plasmodia with simple nuclei are rare. The nuclei in such a case are large and show a diffused chromatic network. They are rarely seen in division. On the other hand, uninucleated or paucinucleated schizonts are very frequently noted. Their form vary greatly. The origin of the double nuclei is very difficult to trace. The nuclear division is rather complex. The two nuclei fuse into one forming a zygote. The sporonts are larger than those of *T. multispora.* The first nuclear division of the sporont is less perfect than that of *T. multispora.* The nuclear filament of the sporont simply condenses at the poles. The second division follows it immediately. In this, two granules, one at each pole, are connected by an achromatic filament which remains for a long time. The third and the last division is similar to that of *T. multispora.* The cytoplasm of a young sporont is uniformly alveolated and does not show any differentiation in its contents. The number of sporoblasts formed in each sporont is variable, but is eight in the majority of cases (Fig. 540). Some sporoblasts seemed to become free under certain conditions and become plasmodia or binucleated schizonts by division of the nuclei. Because of this peculiar change there is no definite arrangement of the various stages of the microsporidian in the tumor.

Spore: Lutz and Splendore: Regularly oval with almost always a clear vacuole at one extremity. Length 5 to 5.5μ, breadth 3 to 3.5μ. When the spores are kept in a moist chamber for a few days, the refractivity of the spore decreases and the latter possesses a paler appearance, the vacuole becoming smaller. The filament is extruded and measures 185 to 212μ in length.

Strickland: Numerous microspores (Fig. 537) and a few macrospores (Fig. 538) were seen. Microspores are oval and possess a thick membrane. A large vacuole is recognizable in the fresh state at the broader end and a smaller one invisible in the fresh state is located at the smaller tip. The nucleus, single or double, is situated close to the latter vacuole. On one occasion two dividing nuclei were noticed. The polar capsule is sometimes distinguishable in the spores stained deeply with hematoxylin, projecting through the dense protoplasm into the large vacuole. The filament is extruded under the action of iodine solution. It is not so easily detached from the spore as that of *T. bracteata*. When detached, it shows a basal knob (Fig. 539). After the extrusion of the filament, the spore contents lose the regularity. It seemed that the polar capsule shrunk and become attached to one side of the spore, while the protoplasm and the nuclei settled down into a small space at the narrow end of the spore. Length 5.8 to 6.6μ, breadth 3.5μ, length of the polar filament 170 to 220μ.

Macrospores are very few in number. This may probably be due to their abnormal formation, for the shell appeared much thicker than that of the microspore. Iodine failed to cause filament extrusion of this kind of spores.

Debaisieux and Gastaldi Size varies (Fig. 541). Average dimensions are 7μ long by 3.5μ broad. Macrospores are very numerous (Fig. 542).

Remarks: See the remarks for the last species.

THELOHANIA MULTISPORA (Strickland 1913)
Debaisieux et Gastaldi 1919
[Figs. 543-548, 765]

1913	*Glugea multispora*	Strickland	1913 : 75-77
1919	*Thelohania multispora*	Debaisieux and Gastaldi	1919 : 189-192

Habitat: Fat body ot the larvae of *Simulium vittatum*, *S. bracteatum* (Strickland) and *S. maculata* (Debaisieux and Gastaldi).

Strickland stated that the microsporidian appeared as small round white masses often measuring as much as 1 mm. in diameter. They may be scattered irregularly throughout the body (Fig. 765) which is not greatly swollen by their presence. Rarer in occurrence compared with *T. bracteata* and *T. fibrata*.

Debaisieux and Gastaldi collected the larvae from January to March and from October to December. The microsporidian is very rare, being found only twice. The tumors are multiple and regular. In one case where a relatively young infection was studied, different stages of the parasite were disposed regularly; the younger stages around the periphery, then a mass of numerous sporoblasts in spherical masses and in the center the isolated spores. In another case where the infection seemed more advanced, no free spores were noticed, but mature spores were grouped in small masses, and there was no regular arrangement as stated above for the tumor. The host nuclei are rarely found in the tumor; but when found they are very voluminous. The tumor is separated from the surrounding tissues by a membraneous layer.

Locality: The United States (in the vicinity of Boston, Mass.) and Belgium (in the vicinity of Louvain).

Vegetative form: Strickland: The sporont (Fig. 543) found around the edge of "myxosporidium" (enlarged host tissue), are rounded bodies measuring 9μ in diameter. The chromatic substance is very diffuse and spreads in a network throughout the cell. The nucleus divides many times (Fig. 544) and at each division the cytoplasm condenses between the newly formed nuclei to form a fine membrane separating them. Usually the sporont retains its globular form during its division. As the division advances, the individual cell assumes a polygonal, usually hexagonal form (Fig. 545, 546). The septa are less pronounced toward the center of the pansporoblast as was seen in sections. When a number of nuclei, varying from 30 to 60, have thus been formed and surrounded by a membrane, the pansporoblast becomes enlarged and each of the numerous uninucleated sporoblasts assumes a more globular shape. The changes in the sporoblast were not observed. The masses of newly formed spores are held together by a quantity of surplus cytoplasm which does not stain so deeply as does that of the spore (Fig. 547). The spores are scattered irregularly throughout the surplus protoplasm.

Debaisieux and Gastaldi: The youngest stage is uninucleated. Nuclear division is essentially alike both in uninucleated and plasmodial forms. After a variable number of division of the nuclei of the plasmodium, they become distributed at the periphery and become surrounded by isolated cytoplasmic bodies. The nuclei grow and then divide. The two parts remain joined or become separated from each other. The cytoplasm of the plasmodium becomes chambered more sharply. The two portions of the diplocaryon fuse and form a single nucleus. This stage is considered as a zygote with a nucleus formed of intermingled filaments. The zygotes develop into sporoblasts synchronously and independently. The successive nuclear divisions are typical to this species. There are to be seen very distinct achromatic spindle fibers. Four filaments which converge at the poles are seen. The chromatic elements are numerous granules; the number was not definitely counted, but probably was about eight at the equatorial plane, 18 at anaphase and 8 or 9 at telophase. During the second division the number of the granules seems to undergo reduction: about 8 at equatorial plate and more than 2 during anaphase and telophase. It is estimated from each zygote eight spores are formed. Further changes in the spore formation could not be made out due to the staining condition. At one end, the sporoblast contains a large nucleus. The intersporoblastic chromatic substances are apparently used for the formation of the spore membrane.

Spore: Strickland: Elliptical and less pronouncedly ovoid than that of *T. fibrata*. On treating with iodine, the filament is extruded. When the filament is extruded, the contents of the spore have the same aspect as was mentioned in the two preceding species. The fluid contents of the vacuole are swollen by the iodine taken up and the as yet undiscovered function of the vacuole may have some connection with the ejection of the filament. Length 4μ, breadth 2.5μ, length of polar filament 40 to 60μ (?).

Debaisieux and Gastaldi: Spores are either completely isolated or grouped in masses (Fig. 548). Length 4 to 5μ, breadth 3μ. No macrospores are found.

THELOHANIA OVATA Dunkerly 1912
[Figs. 549-552]

1912	*Thelohania ovata*	Dunkerly	1912 : 136-137

Habitat: *Homalomyia scalaris*. The author found the parasite in a Giemsa smear of the teased-up rectum of a host individual.

Locality: Scotland.

Vegetative form: Isolated meronts were found with 1, 2, 4 or more nuclei and some of these were apparently budding off uninucleated bodies which may become either meronts or possibly sporonts (Fig. 549). Each sporont forms eight sporoblasts which could be seen in various stages of development (Figs 550, 552), but early divisions were not clearly seen.

Spore: The spore has at first an almost colorless cytoplasm and a mass of material at either end which stains red with Giemsa. It is clearly noted that one of these masses is purplish red, while the other is bright red color. The deeply staining circular mass often seen, is probably the nucleus of the sporoplasm and the pink vacuole is the polar capsule. Macrospores (Fig.

552) elliptical and measure 6 to 7μ long, while the microspores (Fig. 551), a few groups, measure about 4μ long.

Remarks: Compare with *Octosporea monospora.*

THELOHANIA CORETHRAE Schuberg et Rodriguez 1915
[Figs. 553-557, 755, 756]

1915	*Thelohania corethrae*	Schuberg and Rodriguez	1915 : 122-132

Habitat: In the oenocyte of *Corethra* (*Savomyia*) *plumicornis.* Infected larvae were found only in a particular cement basin. Two similar basins and two ponds without cement covering, all only a few meters away from the infected basin, were absolutely free from any infected larvae. The infected larvae were kept in an aquarium, but died gradually. Normal larvae, kept alive in the same aquarium until the next spring, showed no sign of infection. The contents of the aquarium were put into the basin in the spring, but did not bring about any new infection. The infected larvae appeared white instead of being transparent as in the normal condition. The parasites were indicated by one to many spheres under the air-sac in the thorax and also in the 6th, 7th and 8th segments of the abdomen. The spherical mass of the parasite was not the cyst, but the hypertrophied cell with hypertrophied nucleus of the host tissue. In the heavily infected host cell the cytoplasm is not visible and the cell membrane has the appearance of a cyst membrane.

Locality: Germany (Berlin-Dahlem; October, November).

Vegetative form: The youngest intracellular stage which lies in the cytoplasm of the oenocyte, is a small, binucleate spherical form (Fig. 553). In some cases, a single nucleus seems to occur in it. The schizonts divide by binary fission, the nuclei being placed at the ends (Fig. 553). The second division takes place at right angles to the first, producing a cross-shaped group of four daughter cells, each with two nuclei arranged in pairs. These divide again, and form an eight cell stage which still remain unseparated (Fig. 554). They become detached and later set free. The nucleus divides amitotically, though this cannot be determined clearly owing to the smallness of the object. The ratio of the volume of the body between the youngest schizonts and the final stage is 1 : 8. The repetition of the schizogony of the young schizonts may probably occur although it was not actually observed, otherwise the heavy infection cannot be explained.

Sporogony starts with uninucleated spherical sporonts. The nucleus of a sporont is probably formed by autogamous union of the two nuclei of the former stage. The cytoplasm is highly vacuolated compared with the compact cytoplasm of the schizont. The nucleus divides amitotically. Two daughter nuclei are often seen connected by a cytoplasmic thread, giving the appearance of indirect nuclear division (Fig. 555). The nuclei divide further without being accompanied by the complete separation of the cytoplasm, similar to the corresponding stages in schizogony. The ultimate form of the division is an eight cell stage attached at first to a dense central protoplasmic mass (Fig. 556) and later breaking up into eight spores. Spore formation could not be followed.

Spore: Pyriform (Fig. 557). Apparently similarly built as that of *Plistophora longifilis.* The sporoplasm is in a ring form and contains a single nucleus. The polar capsule was not observed. Dimensions not given.

Remarks: A similar species was observed by Lutz and Splendore which is here provisionally given the name *Th. brasiliensis*. No attempts are made to compare them because of the inadequacy of the descriptions.

THELOHANIA sp. Bresslau 1919

1910	*Thelohania* sp.	Bresslau and Buschkiel	1919 : 327

Habitat: In a larva of *Culiseta* (*Theobaldia*) *annulata.*

Locality: Germany (Frankfurt a. M. ?).

The author simply states that he saw chromosomes in the sporulation division.

Remarks: According to the view of Bresslau, the species is provisionally listed here.

THELOHANIA sp. Nöller 1920

[Fig. 558]

1920	*Thelohania legeri*?	Nöller	1920 : 187

Habitat: Adipose tissue of a larva of *Aëdes* (*Culex*) *nemorosus.*

Locality: Germany (near Hamburg; Spring).

Vegetative form: Nöller gives figures representing stages in the sporogony of this species, in which very distinct nuclear changes are noticed (Fig. 558).

Spore: Mature spores were not observed.

Remarks: Nöller maintained that the species is probably *T. legeri*, an exclusive microsporidian parasite of anopheline mosquitoes. I am inclined to think that Nöller had a species very close to, if not identical, with *T. opacita*, a typical parasite of culicine mosquitoes. Since no dimensions are given, the species is separated from other known forms.

THELOHANIA sp. Iturbe et Gonzalez

1921	*Thelohania legeri* (?)	Iturbe and Gonzalez	1921 : 5

Habitat: In the larva of *Aëdes pipiens.*

Locality: Venezuela.

Remarks: Comparison with known species parasitic in mosquitoes is impossible, since the description is inadequate.

THELOHANIA OPACITA Kudo 1922

[Figs. 560-570, 749, 750, 763]

1922	*Thelohania opacita*	Kudo	1922 : 75-76
1924	*Thelohania opacita*	Kudo	1924a (in press)
1924	*Thelohania opacita*	Kudo	1924c (in press)

Habitat: The adipose tissue of the larvae of *Culex testaceus* (*C. apicalis*), *C. territans* and *C.* sp.

In all, three of the first host species (New York; August), ten of the second host (one from Alabama; August, and the rest from New York; September) and one *C.* sp. (Georgia; September) have been found to suffer from the infection of the present microsporidian. All were more or less heavily infected and showed typical symptoms of the disease—opacity of the body and decrease in activity. They succumbed to death before pupation much more quickly than normal larvae. Infected pupae or adults were not found.

Locality: The United States (New York, Alabama, Georgia).

Vegetative form: Schizogony is binary fission (Fig. 559) and the process is quite similar to that of *T. legeri*. The nuclei of the dividing schizonts are typically vesicular. The final products of the schizogony are binucleated bodies with strikingly deeply staining cytoplasm (Fig. 560). The two nuclei fuse to form uninucleated sporonts (Fig. 560). The three successive nuclear divisions during the process of sporogony are similar to those of the corresponding stages of *T. legeri* (Figs. 561 to 564). The extranuclear chromatin granules are not so abundant as in the case of the latter species. The sporont is typically octosporous (Figs. 565, 567), but frequently tetrasporous (Fig. 566). Consequently there are larger and smaller spores formed. No significance is attached to this dimorphism of the spore, other than the abnormality in sporulation.

Spore: Broadly ellipsoid and circular in cross-section (Fig. 568). In some spores, one end is more rounded than the other, while in others the ends are somewhat equally truncated. A rounded triangular, oval or circular clear space is always present in mature spores, while the other part of the spore is uniformly and finely granulated. When the spores are subjected to mechanical pressure the filament becomes extruded from the side (Fig. 749) or from the tip. In moderately pressed spores, there is a longitudinal line distinctly visible on the spore membrane (Fig. 570, 750). This is probably a sutural line of shell-valves. Fresh spores measure 5.5 to 6μ long by 3.5 to 4μ broad. Fixed spores measure 4.5 to 5.5μ by 3.3 to 4μ. The filament is 90 to 110μ long. The large spores measure 8 to 8.5μ long by 4.5 to 5.5μ broad in fresh conditions and 6.5 to 7.5μ long by 5 to 5.2μ broad in fixed state The filament reaches 200μ in length

THELOHANIA RENIFORMIS Kudo et Hetherington 1922
[Figs. 679-683]

1922	*Thelohania reniformis*	Kudo and Hetherington	1922 : 130-132
1924	*Thelohania reniformis*	Hetherington	1924, fig. 45

Habitat: In the epithelial cells of the intestine of *Protospirura muris*, parasitic in the digestive tract of *Mus musculus*. The microsporidian was found in the gut epithelial cells of the worm throughout its entire length save the very extremities and isolated spores as well as those still enclosed in the sporont membrane were also noticed in the lumen of the organ. Other organs of the host were free from the infection. The infection was very light.

Locality: The United States (Illinois).

Vegetative form: The young schizont is a small oval or rounded mass of finely granulated cytoplasm containing a single nucleus and is embedded in the cytoplasm of the host cell. As it grows in size, there becomes visible a narrow clear space between it and the surrounding cytoplasm. The nucleus is a deeply staining chromatin granule as is ordinarily the case. Schizogony is binary fission and nuclear division is amitotic (Fig. 681). In all the worms which the authors examined, they did not see the condition commonly found in Microsporidia where the schizogony is repeated rapidly and actively so that the host cell becomes crowded by the parasite. There seem to exist some inhibiting factors in the present host species to counteract the activity of the microsporidian.

The schizonts become the sporonts. Both the nucleus and the body become larger and the latter vacuolated. The nucleus divides three times, producing eight nuclei. Thus eight sporoblasts are formed in each sporont. Each sporoblast develops into a spore. The sporont membrane remains visible for a long time (Figs. 682, 683).

Spore: Kidney-bean shaped (Fig. 679); circular in cross-section. The shell is relatively thin and therefore the spores are not so refractive as *Nosema bombycis*. The contents of the spore are divided into two regions: near one end which is frequently more rounded than the other, there is a rounded clear area, while the remaining part of the spore is finely granulated. Length 3 to 4μ, breadth 1.5 to 1.8μ. Under the action of mechanical pressure the filament is extruded. The latter measures 45 to 55μ in length (Fig. 680).

THELOHANIA MUTABILIS Kudo 1923
[Figs. 684-687]

1923	*Thelohania mutabilis*	Kudo	1923 : 22-23

Habitat: Adipose tissue of the nymph of *Ameletus ludeus*. The insects were collected from a small overflow stream of a spring in the woods. Four out of 32 nymphs examined were infected. The infected nymphs did not show any noticeable decrease in activity, although slight opaque coloration was recognized in the infected area of the body.

Locality: The United States (Pennsylvania).

Vegetative form: Young schizonts are uninucleated. Schizogony is either binary fission or multiple division, producing two, four or eight daughter individuals (Fig. 684). The division seems to be repeated. The nucleus is compact and its division is amitotic. Each schizont grows into a large body, a sporont, whose nucleus becomes vesicular. After three divisions of the sporont nucleus, eight sporoblasts are formed (Fig. 685). There seems to be a considerable variation in the size of the pansporoblasts at the end of sporoblast formation. A small number of tetrasporoblastic sporonts is found.

Spore: Oval, elongated ovoid or pyriform in form; circular in cross-section (Fig. 686). Less refractive than *Nosema bombycis*. Fresh spores may show a small rounded clear space at one end and measure 3.8 to 5.5μ long by 2.5 to 3μ broad. Length of filament is 70μ.

THELOHANIA BAETICA Kudo 1923

[Figs. 688-693]

1923	*Thelohania baetica*	Kudo	1923 : 23-24

Habitat: The adipose tissue of the nymphs of *Baetis pygmata* (?). Five out of twenty six nymphs were parasitised by the microsporidian. The infection was light; no particular coloration or decrease in activity of the infected host insects was noticeable, although the host nuclei showed a hypertrophied condition.

Locality: The United States (New York; August).

Vegetative form: Octosporoblastic sporonts were found exclusively. The peculiar characteristic of the present form is that the nucleus is relatively large throughout different stages of schizogony (Figs. 688, 689) and sporogony (Figs. 690, 691).

Spore: Oval (Fig. 692). Highly refractive. Spore membrane thick. A clear vacuole rounded triangular in form may be seen at one end. Fresh spores: Length 2 to 4.5μ, breadth 2.5μ. The extruded filament is 100μ long.

THELOHANIA OBESA Kudo 1924

[Figs. 731-737]

1924	*Thelohania obesa*	Kudo	1924a (in press)

Habitat: The adipose tissue of a larva probably of *A. quadrimaculatus*. A single larva was found dead when brought into the laboratory. It was fully grown and darkly colored. The infection was very heavy. Numerous anopheline larvae infected by *T. legeri* were collected from the same locality.

Locality: The United States (Georgia; September).

Vegetative form: The schizont was not noticed. The uninucleated sporont (Fig. 731) is probably formed by a fusion of two nuclei in a binucleated schizont. It measures 6 to 7μ in average diameter and possesses numerous deeply staining grains in its peripheral part. The sporont nucleus undergoes usually three divisions which finally develop into eight spores (Figs. 732 to 736). The fully formed octosporous sporont, slightly flattened in thick smears, measures 9 to 10μ in largest diameter. Compared with *T. legeri*, the deeply staining bodies which appear in the sporont during its development, are much larger in size and more conspicuous. They, however, disappear gradually as the spores are formed, probably being used for the formation of the spore membrane.

Spore: Broadly oval with somewhat flattened ends (Fig. 737). Membrane is moderately thick and refractive. Some spores show a rounded clear area near one end, while others are uniformly filled with finely granulated contents. Fixed spores measure 4 to 4.5μ long by 3 to 3.5μ broad. The filament is 45 to 55μ long.

THELOHANIA PYRIFORMIS Kudo 1924

[Figs. 738-742]

1924	*Thelohania pyriformis*	Kudo	1924a (in press)

Habitat: The adipose tissue of an anopheline larva (*A. crucians* or *quadrimaculatus*). The host was fairly well grown and showed the typical symptoms of a heavy infection by its opaque yellowish coloration and great inactivity. Its body was completely filled with mature spores. The death of the host was probably due to the microsporidian infection.

Locality: The United States (Georgia; September).

Vegetative form: Schizogonic stages were not noted. The sporont is typically octosporous (Figs. 738, 739). The membrane is indistinct and eight developing sporoblasts become separated from one another.

Spore: Pyriform (Fig. 740); and circular in cross-section. Moderately refractive. At one end which is broadly rounded, a rounded or oval vacuole is present. This vacuole is a part of the sporoplasm uncovered by the coiled polar filament (Fig. 741). The fresh spores measure 4.8 to 5.4μ long by 2.7 to 3μ broad. The sporoplasm contains a single nucleus and is located near the broad extremity of the spore. The extruded filament measures 70 to 100μ long (Fig. 742). Fixed and stained spores measure 3.5 to 4μ long by 2 to 2.8μ broad.

THELOHANIA ROTUNDA Kudo 1924

[Figs. 743, 744]

1924	*Thelohania rotunda*	Kudo	1924a (in press)

Habitat: The fat body of a young larva of *Culex leprincei*. The posterior portion of the larva showed some masses of the microsporidian, which were visible with unaided eye, although the infection was moderate.

Locality: The United States (Georgia).

Vegetative form: The spherical octosporous pansporoblast measures 6.5μ in diameter (Fig. 743).

Spore: Broadly ovoid or subspherical (Fig. 744). Spore membrane relatively thick. Highly refractive. An oval clear space at one end. Fixed and stained spores measure 2.5 to 3μ long by 2.3 to 2.7μ.

THELOHANIA MINUTA Kudo 1924

[Figs. 745-748]

1924	*Thelohania minuta*	Kudo	1924a (in press)

Habitat: The larvae and pupae of *Culex leprincei* and *C.* sp. Seven infected hosts were observed. Five larvae of the first mentioned species showed more or less heavy infection. One of them pupated and died in that stage. The sixth larva lived, pupated, but died before becoming an adult. In the case of heavy infection, the insects showed similar behavior and appearance as those attacked by *T. opacita*. Although the primary seat of infection is the fat body, in the dead pupa the infection extended from that tissue into the ventral nerve chord. The muscles also seemed to be infected.

Locality: The United States (Georgia).

Vegetative form: Octosporous pansporoblasts were abundantly noticed (Fig. 745).

Spore: Ovoid in shape (Fig. 747), equally rounded at both extremities. It is circular in cross-section (Fig. 748). The contents are finely granulated. In many fresh spores a faintly visible fine strand connects the contents with one of the ends. Fresh spores measure 3.5 to 3.9μ long by 2.4 to 2.7μ broad. Fixed spores measure 2.5 to 3.3μ long by 1.5 to 2μ broad.

Genus STEMPELLIA Léger et Hesse 1910

The characters of the genus are described on page 67.

Type species: *S. mutabilis* Léger et Hesse 1910.

STEMPELLIA MUTABILIS Léger et Hesse 1910

1910	*Stempellia mutabilis*	Léger and Hesse	1910 : 412-413

Habitat: Fat body of the nymph of *Ephemera vulgata* L.

Locality: France.

Vegetative form: Numerous individuals present in cysts which are spherical or ovoidal, varying in size with the largest diameter of about 120μ. They are scattered in the adipose tissue and are surrounded by a somewhat thick envelope of the connective tissue of the host, which is a reaction product against the microsporidian. In the cyst numerous sporont stages of various phases of development are noted. Different individuals develop different number of spores that is octosporous (Thelohania type), tetrasporous (Gurleya type), rarely disporous (Perezia type) and monosporous (Nosema type). Ultimately the cyst falls into the general body cavity.

Spore: Generally ovoidal; some pyriform in tetrasporous type. Size variable 2 to 6μ. Isolated spores are voluminous.

STEMPELLIA MAGNA Kudo 1920

[Figs. 571-596, 769, 770; Textfig. I]

1920	*Thelohania magna*	Kudo	1920 : 178-182
1921	*Thelohania magna*	Kudo	1921a : 156-166, 176, 177 1921c : 73-74
1924	*Stempellia magna*	Kudo	1924b (in press)

Habitat: Adipose tissue of the larvae of *Culex pipiens* and *C. territans*. Six out of 38 of the first named host species and 43 out of 290 larvae of the second species were more or less heavily parasitized by the microsporidian. No infected pupae or adults were found. The heavily infected larvae, particularly those of *C. territans* were characterized by opaque whitish coloration and decrease in size and activity of the body. Rearing experiments showed that the infected host larvae succumbed to death more quickly than the normal larvae under similar conditions.

Experimental infection *per os* showed that the liberated amoebulae first begin their intracellular existence in the fat bodies surrounding the posterior portion of the mid-gut and also the tracheae in that region. Spores were not formed four days after the spores were swallowed.

Locality: The United States (Illinois, Pennsylvania).

Vegetative form: The emergence of the sporoplasms as amoebulae starts between 6 and 24 hours after the ingestion of the spores (Fig. 571). The amoebulae are uninucleated. Intracellular schizonts are found as early as 24 hours after feeding on the infected material in the situation mentioned above. Round in form, such a stage shows reticulated cytoplasm and a large and compact nucleus, the chromatin granules appearing to accumulate in a peripheral layer (Fig. 572). The schizonts grow and by binary fission multiply in number (Figs. 573, 574). In the somewhat advanced stage of schizogony the nuclei divides twice without cytoplasmic constriction so that tetranucleated forms are produced (Fig. 575). The schizonts grow and the nuclei divide, which apparently starts with the division of the karyosomes. Thus oblong schizonts possessing three to eight nuclei are formed (Figs. 577 to 579). These multinucleated forms seem to divide ultimately into binucleated bodies (Fig. 580). As the two nuclei of the schizont come in a close contact, each karyosome buds off a small chromatin granule which seems to be extruded into the cytoplasm (Fig. 581). The nuclear membranes between the two nuclei disappear, while the two karyosomes become fused into one. The chromatin grains that were thrown out into the cytoplasm seem to divide further. This uninucleated body is the sporont (Fig. 582) and gives rise to spores through the following changes: After some growth in size of the uninucleated sporont, its karyosome divides into two which become separated by a nuclear wall and the two nuclei are formed. The nuclei become separated from each other and locate themselves near the opposite extremities. A septum appears in the cytoplasm, and two sporoblasts are formed. Each sporoblast develops into a spore (Fig. 583). Frequently the two daughter nuclei divide once more (Fig. 584). The division begins with that of the karyosome, a strand persisting usually between the latter. Thus a sporont with four nuclei is formed; its cytoplasm as in the case of the disporoblastic sporont, divides into four sporoblasts (Fig. 585), each of which develops into a single spore. Less frequently a sporont nucleus divides three times, thus producing eight sporoblasts (Fig. 586) which later develop into eight spores. Quite frequently the sporont when discharged into the body cavity of the host transforms into a sporoblast and later into a single spore without any division as stated in the above instances. This process is wholly responsible for the production of abnormally large spores. The cytoplasm of the sporont is more vacuolated and less deeply stained than that of the schizont of which mention was made in many other species. The sporoblast which varies greatly in size as the natural sequence of the difference in its production, is rounded or oval in form (Fig. 587). It has a nucleus composed of peripheral chromatin grains and a karyosome. There are to be seen one or more chromatin grains near one end. The nucleus moves toward the other end of the sporoblast while deeply staining granules appear in the clear space at the other extremity. These granules become smaller in size and larger in number as the filament is formed which probably indicates that they are used for the formation of the polar filament (Fig. 588). When nearly formed, the spore contains the following structures (Textfig. B 8): the sporoplasm with one nucleus near the rounded end and the coiled polar filament at the other part of the spore (Fig. 591).

Spore: In the fresh state, the fully formed spore is elongated pyriform, often slightly bent toward one side (Figs. 589, 590). In cross-section it is circular. One end which is ordinarily called the posterior end is rounded, while the other end is less rounded though not attenuated. The spore is moderately refractive and presents somewhat varied aspects. In a large number of spores, there is to be seen an oval, cap-shaped or rounded area, through which a fine protoplasmic strand sometimes runs transversely, while the other part is finely granulated (Fig. 590) and show fine irregular lines of a coiled filament. In some spores, there is no clear space such as just noted and in others which apparently possess a thin membrane numerous transverse cytoplasmic strands are to be seen (Fig. 589). Fresh spores measure 12.5 to 16.5μ long by 4 to 4.6μ broad. Some abnormally large spores reach 25μ long by 10μ broad, which are without doubt the products of monosporous sporogony. When the spores are treated with methylene blue M.P., there appears a deeply stained round body surrounded by less deeply staining cytoplasm which is held as the sporoplasm, while in the remaining part, an irregular network becomes distinctly visible which is doubtlessly the polar capsule with its coiled filament (Fig. 591). In large spores, the polar capsule does not seem to be present, in which case the spore is almost exclusively occupied by transverse lines (Fig. 592), the filament; while in smaller ones, the above stated condition is distinctly recognized. The polar filament when extruded under the influence of mechanical pressure measures 350 to 400μ long. Except its base, the filament is uniformly fine, the diameter being less than one-third of a micron (Figs. 593-595). When the pressed spores are mounted in a Lugol and gum-arabic mixture and left for two days, the filament is considerably and uniformly thicker than a Fontana stained one, as a result of the swelling due to the medium (Fig. 596). Very rarely as in the other species which the author studied, one sees a thick point at the extremity of the extruded filament, which is probably due to the broken condition of the structure during the process of extrusion and to the spreading out of the material which compose the filament.

Genus DUBOSCQIA Pérez 1908

The characters of the genus are described on page 67.
Type and only species: *D. legeri* Pérez 1908.

DUBOSCQIA LEGERI Pérez 1908

1908	*Duboscqia legeri*	Pérez	1908 : 631-633
1909	*Duboscqia legeri*	Pérez	1909 : 17-19

Habitat: General body cavity of *Termes lucifugus* Rossi.

Locality: France (Landes de Gascoque).

Vegetative form: The microsporidian, always found in small numbers, two or three at the most, is a white spherical mass, 500μ in diameter and floats in the general body cavity. The external surface is limited by a plasmodial layer. The nuclei are branched and budding and are rich in chromatic substances. These nuclei exceed 60μ in length. In the central portion of the body, small spherical sporonts, 2.5μ in diameter with a small chromatin granule, are present. The sporonts grow later into ovoidal pansporoblasts, 12μ by 7μ, which develop 16 spores simultaneously. The sporulation is comparable with that in the genus Thelohania. A thin membrane surrounds the group of the spores.

Spore: Oval. Length 5μ, breadth 2.5μ.

Remarks: It is most probable that the so-called floating parasitic mass is none other than the infected host cell.

Genus PLISTOPHORA Gurley 1893

The characters of the genus are described on page 67.

Type species: *P. typicalis* Gurley 1893.

PLISTOPHORA TYPICALIS Gurley 1893

[Figs. 597, 598]

1890		Thélohan	1890 : 203, 212
1891		Thélohan	1891 : 27
1891		Pfeiffer	1891 : 113
1892	Glugéidée	Thélohan	1892 : 174
1893	*Pleistophora typicalis*	Gurley	1893 : 410
1894	*Pleistophora typicalis*	Gurley	1894 : 194-195
1895	*Pleistophora typicalis*	Thélohan	1895 : 361
1899	*Plistophora typicalis*	Labbé	1899 : 108-109

Habitat: The muscles of *Cottus bubalis*, *C. scorpius*, *Blennius pholis* and *Gasterosteus pungitius*.

Locality: France (Concarneau, Roscoff, Rennes).

Vegetative form: Gurley: Thélohan observed between the host fibrillae small masses of protoplasm (Fig. 597), each with a distinct membrane and nuclei. These masses were 4μ long by 2.5 to 3μ broad. Pansporoblasts spherical, surrounded by a thin membrane, and 15 to 18μ in average diameter.

Thélohan (1895): Pansporoblasts (vesicles) spherical, embedded in the host fibrillae and 25 to 35μ in diameter. A large and inconstant number of spores is contained in them.

Spore: Gurley: Ovoid, resembling that of *Glugea anomala*. A polar capsule with a polar filament is present. Length 3μ, breadth 1.5 to 2μ.

Thélohan (1895): The polar filament is very long when extruded under the action of iodine water (Fig. 598). Length 5μ, breadth 3μ, length of the extruded filament is 65 to 75μ.

PLISTOPHORA OBTUSA (Moniez 1887) Labbé 1899

1863		Leydig	1863 : 187
1887	*Microsporidia obtusa*	Moniez	1887 : 185
1887	*Microsporidia ovata*	Moniez	1887 : 185
1887	*Microsporidia acuta* (part.)	Moniez	1887 : 185
1887	*Microsporidia elongata*	Moniez	1887 : 185
1887	*Microsporidia incurvata*	Moniez	1887 : 185

1887	*Nosema parva* (part.)	Moniez	1887a : 1313
1895	*Glugea leydigii* (part.)	Pfeiffer	1895 : 72-73
1899	*Plistophora obtusa*	Labbé	1899 : 109
1905	*Nosema incurbata*	Mesnil	1905 : 403

Habitat: The body cavity of *Simocephalus retulus*, *Polyphemus oculus*, *Chydorus sphaericus*, *Daphnia pulex*, *Ceriodaphnia reticulata*, *Moina rectirostris*, *Daphnia longispina* and *D. obtusa*.

Locality: France and Germany.

Moniez described various forms as follows: *Microsporidia obtusa* in *Simocephalus retulus* and *Daphnia reticulata*. Spores are obtuse and broad, possessing always a clear body (nucleus or vacuole?). Length 4μ, largest breadth 2.5μ. *M. ovata* in *Simocephalus retulus* and *Chydorus sphaericus*. Spores are perfectly oval. The clear body is rarely visible. Length not exceeding 3μ. *M. elongata* in *Simocephalus retulus*. Spores are elliptical. Length 5μ or more, breadth 2μ. *M. acuta* in *Daphnia pulex*. One end of the spore highly pointed. Length 5μ, breadth 2μ. *M. incurvata* in *Daphnia pulex*. The extremities of the spores are slightly different; regularly curved. Length 5μ, breadth 2μ. *Nosema parva* in *Cyclops* sp. Sporogenous mass voluminous. Spores oval with a clear body at one end. Length 3.5μ, breadth 2μ.

Labbé considered all these species of Moniez as belonging to one species giving the name, *P. obtusa*. Inasmuch as the original descriptions were inadequate I agree with Labbé in bringing them together under a single specific name.

Vegetative form: Pansporoblasts spherical or subspherical and contain eight to thirty two or more spores.

Spore: Oval or pyriform; slightly curved and with a vacuole. Macrospores and microspores. Length 3 to 5.5μ, breadth 1 to 3μ.

PLISTOPHORA MIRANDELLAE Vaney et Conte 1901

1901	*Plistophora mirandellae*	Vaney and Conte	1901 : 644-646

Habitat: The ovary and ovum of *Alburnus mirandella*.

Locality: France (Lyon?).

Vegetative form: The infected ovule presents a large number of cavities in which one sees rounded masses, each possessing a large vacuole in the center. It is amoeboid in form. A single nucleus is located in the cytoplasm. In the interior of the body, numerous spores are formed. The small cyst with a resistant envelope shows a dark color due to the accumulation of the spores in the central region. The cyst contains microspores. The larger cyst surrounded by a less resistant envelope, has a clear appearance with scattered spores which are always macrospores.

Spore: Ovoidal. Macrospores are scattered in the host connective tissue and measures 12μ long by 6μ broad. A vacuole is present at one end.

The polar filament, very long, is extruded under the influence of iodine water. Microspores measure 7.5μ long by 4μ broad. Their structure seems to be similar to that of the macrospores. Cysts of microspores remain intact. The macrospore serves for autoinfection, while the microspore infects new host fish.

PLISTOPHORA ACERINAE Vaney et Conte 1901
[Figs. 599, 600]

1901	*Plistophora acerinae*	Vaney and Conte	1901 : 105-106

Habitat: The mesentery of *Acerina cernua*. A single infected host fish was found.

Locality: France (Lyon; February.)

Vegetative form: The microsporidian forms a whitish elongated mass of about 3 mm. in length attached to the mesentery. The mass contained sporoblasts and spores. The sporoblasts are rounded in form. The membrane of young pansporoblasts is thin, while that of older ones in which the spore formation has begun is thick. The pansporoblasts varied in size according to the number of spores they contained.

Spore: Ovoidal (Fig. 600). When stained the spore shows two regions in its contents: one, a large portion, always stained deeply by carmine, methyl-green, Borel blue, etc., corresponds probably to the polar capsule; the other, much smaller and usually unstained, is without doubt the vacuole. The filament was extruded when the spores were treated with iodine water. Length 3μ, breadth 2μ, length of the filament measured from the figure given by the authors is 15μ, although they stated that the structure was very long.

PLISTOPHORA STEGOMYIAE (Marchoux, Salimbeni et Simond 1903) Chatton 1911
[Figs. 601-607]

1903	*Nosema stegomyiae*	Marchoux, Salimbeni and Simond	1903 : 714-728
1903	*Nosema stegomyiae*	Simond	1903 : 1335-1337
1906	*Nosema stegomyiae*	Marchoux and Simond	1906 : 18
1911	*Pleistophora stegomyiae*	Chatton	1911 : 664

Habitat: The larva and imago of *Stegomyia fasciata*.

Marchoux, Salimbeni and Simond found in 1902 from January to June, 10 infected hosts out of 300 adults (female) examined. In 1903 the authors examined in the same months more than 200 adults and found three infected individuals. The seats of infection in the adult insect are the stomach, esophagus, air-sac, coelom, the Malpighian tubules, ovary, ovum, thoracic muscles, the large ganglion and the tracheal epithelium. In the larvae which were less frequently infected than the imago, vegetative forms and colorless spores were observed. The seat of infection is the digestive tract, body cavity, tissues at the posterior part of the body and anal papilla. The new infection takes place through the mouth and eggs, the latter being more frequently met with.

Locality: Brazil (Rio-de-Janeiro).

Vegetative form: The spores are of two kinds which undergo entirely different development. Simond: Development of colorless spores. The spore membrane disappears, leaving a small spherical plasmodium of granular structure (Fig. 605). The plasmodium is immobile, and is attached to the host tissue. Its diameter is about 40μ or larger. At a certain period the body divides into small refractile bodies variable in number (Fig. 606), each of which becomes a spore. At the end of the evolution the plasmodium is a mass of spores (Fig. 607). After becoming free, the spores reach various parts of the body with the movements of the body fluid and repeat the changes. Development of brown spores. Instead of forming a plasmodium, the brown spore transforms into a more or less dark brown thread-like body which is ordinarily simple, but exceptionally with one or two very short branches. It is different from the mycelium of a fungus and is irregular. At the end of the growth it develops into rounded enlargements along the axis, presenting an appearance similar to a chaplet. The length rarely exceeds 20 to 30μ.

Spore: Marchoux, Salimbeni and Simond: Generally reniform, more or less elongated. One extremity is frequently more attenuated than the other, exhibiting a form of a comma, ovoidal or spheroidal. Two kinds. Colorless spores. Reniform (Fig. 603). Highly refractile. Extremities are usually equal in form, sometimes one being more attenuated than the other. Shell double contoured. The cytoplasm is homogeneous in the fresh state. Ordinarily one circular or oval refringent area is present at one end. Length 4 to 7μ, breadth 2 to 3μ. Brown spores. They occur either in a mass of colorless spores or in a mass composed only of brown spores. Chocolate brown or light color. Ovoidal or more or less spherical. The shell is thicker than in the colorless form, is more or less transparent and becomes brownish later.

Simond: The small protoplasmic mass of the colorless spore shows no nucleus in fresh state. Length 3 to 5μ, breadth 2 to 3μ.

Remarks: The typical microsporidian characters of the spore are not established and moreover the development is quite different from that of the rest of the Microsporidia. Hence its microsporidian nature is open to question. Supposing it to be a microsporidian, the species is placed in the genus Plistophora in agreement with Chatton. See also *Nosema stegomyiae.*

PLISTOPHORA SIMULII (Lutz et Splendore 1904)
Debaisieux et Gastaldi 1919

1904	*Nosema simulii α, β*	Lutz and Splendore	1904 : 647
1919	*Plistophora simulii γ, δ, ε*	Debaisieux and Gastaldi	1919 : 196-201

Habitat: The larvae of *Simulium venustum* and *S. ochraceum* (α and β forms) and *S. maculata* (γ, δ and ε forms).

Locality: Brazil and Belgium.

FORM α

Spore: According to Lutz and Splendore the spores are refractive. The size and form vary. Small, almost spherical oval form to short cylindrical ones; one end attenuated and the other rounded. At the latter extremity, a vacuole of variable size is observable. At the former end there is to be seen a distinct foramen through the shell. Through this foramen, the polar filament is extruded. The spore membrane becomes separated into two shell-valves. Frequently large spores are noted which are twice or more the normal size They contain a vacuole, but a filament or foramen in the shell was not recognized The spores measure 5.5 to 8.5μ long by 4.5 to 5.5μ broad. Length of the polar filament is up to 120μ

FORM β
[Fig. 608]

Spore: Lutz and Splendore observe that the spores are narrower than α form, but nearly of the same length Regularly oval or elongated ovoidal. A vacuole is always seen at one extremity. The spore membrane opens into two shell-valves after the polar filament is extruded. Length 45 to 5.5μ, breadth 2.5 to 3.5μ, the filament 50μ long.

FORM γ
[Fig. 609]

Debaisieux and Gastaldi observed as follows: The tumors, single or multiple, are regularly rounded and lobated. The different stages are arranged very irregularly: the young stages at the peripheral portion, then groups of young sporonts, sporonts with developing sporoblasts and spores in groups of 20 to 30, and in the center the mature spores. In the case of a heavy infection this arrangement is not seen. The tumor may or may not be surrounded by a definite membrane, coming in direct contact with the fat body of the host. One sometimes sees a small number of spores or sporoblasts in the adipose tissue cells of the host. In the tumor particularly at its periphery, are seen nuclei which are very much larger than any apparently connected with the microsporidian. They are numerous and contain a large nucleolus. They are probably the nuclei of the host cells.

Vegetative form: The plasmodia are rare. Young and uninucleated stages were observed. They were noted at the periphery of the tumor. Whether or not the uninucleated forms produce uninucleated bodies by fission is not known. Simultaneous division of the nuclei of the binucleated form was abundantly observed. Thus daughter diplocarya are formed. The two nuclei fuse completely into one and a zygote is thus formed. The nuclear division in the sporont is mitotic; in it there is a central achromatic axis running in the direction of the division. The number of nuclear divisions in a sporont seems to be variable, forming 20 to 30 sporoblasts, each of which develops into a single spore. The sporoblast possesses a large nucleus. Later a chromatic granule appears in the cytoplasm whose origin is not clear. A vacuole becomes differentiated at each end of the sporoblast. Into one of the vacuoles the cytoplasm projects, which later becomes condensed at the middle part of the spore in a biconcave band. It is very chromatophilous. The granule develops into a dumbbell form. In the other vacuole the coiled filament is produced. The process is similar to that of *Glugea mülleri* or *G. danilewskyi.*

Spore: Average dimensions 6 to 8μ long by 3.5 to 5μ broad. Some macrospores were seen.

FORM δ
]Fig. 610]

Debaisieux and Gastaldi stated that the tumors were regularly rounded and different stages were arranged regularly in concentric layers in them. The host nuclei, probably of adipose tissue cells, are very numerous and large with several nucleoli. They are often seen undergoing mitotic division.

Vegetative form: The general development is similar to that stated for the last form. The binucleated forms, sporonts and sporoblasts are, however, much larger than in the γ form.

Spore: Dimensions not given. Enormous macrospores were noted.

FORM ϵ

Debaisieux and Gastaldi saw this form only three times. The tumor was colored red. Vegetative form and spores are similar to those of form δ.

PLISTOPHORA VAYSSIEREI (Hesse 1905) Kudo

1905	*Nosema vayssierei*	Hesse	1905a : 917-919

Habitat: Fat body of the nymphs of *Baetis rhodani.* Hesse noted some ten cases of infection out of about one thousand host individuals examined. No external deformity of the host body was present, but the ventral side of the nymphs showed a chalky-white coloration. The nymphs were more frequently infected than the adults, which fact probably indicates that the infection is mortal at least to young nymphs. The microsporidian did not produce any cyst, but was distributed throughout the fat body in a state of irregular diffuse infiltration.

Locality: France (from the running water at Montessaux).

Vegetative form: The pansporoblasts either oval, 9 to 12μ long by 6 to 9μ broad, or spherical, 8 to 10μ in diameter, contain a variable number of spores.

Spore: Pyriform, with a small vacuole, often refringent, at the posterior end and a polar capsule visible in a large number of spores at the anterior extremity. At the latter end there seems to be a stopper which becomes detached itself when the filament is extruded. The extrusion of the polar filament took place under the action of iodine water, sulphuric acid or physiological solution in about thirty minutes. Length 3 to 4μ, largest breadth 1 to 2μ, length of the filament 17 to 19μ.

Remarks: Because of Hesse's statement about the pansporoblast, the species is removed from Nosema and provisionally placed here.

PLISTOPHORA MACROSPORA Cépède 1906

[Figs. 613-616]

1906	*Plistophora macrospora*	Cépède	1906 : 13-15 1906a : 15-16
1916	*Plistophora macrospora*	Léger and Hesse	1916a : 1051-1053

Habitat The muscles of the abdomen of *Cobitis barbatula*. Cépède examined one infected fish, 6 mm. in total length.

Locality: France (in the vicinity of Grenoble).

Vegetative form: Cépède: The tumor was yellowish white and transparent. It was ellipsoidal in form and measured about 3 mm. in diameter, distending the abdominal integument. Its upper margin was 1.5 mm. below the lateral line. The tumor contained pansporoblasts and spores at various stages of development. The sporont in fresh condition is spherical or subspherical and measures 25 to 30μ in diameter. It is provided with a double contoured envelope. The pansporoblasts contain mature spores in a large, but variable, number.

Spore: Cépède: Form variable according to the stages of ripening. Some are distinctly ovoidal and have a clear space at each end; the central portion is occupied by a granular and less refringent cytoplasmic mass and the shell is not so distinctly visible. Others have only one clear space at one end, in which the coiled filament is distinctly visible; the opposite end is occupied by a cytoplasmic mass of finely granular structure. The polar filament is extruded under the action of physiological solution for one hour. The spore presents varied appearances after the filaments are extruded. Some become filled with clear granular contents and show in the interior refringent arch-shaped or circular figures. Others exhibit a large refringent space surrounded by a slightly yellowish pale peripheral zone which is thicker at the extremities. Spores stained with iron hematoxylin show a very similar structure as worked out by Stempell. Fresh spores measure 8.5μ long by 4.25μ broad. The filament is 225μ long.

Léger and Hesse: The polar capsule is present in the central part of the spore, while the sporoplasm is located in a space at the posterior extremity (Textfig. B 6). For the demonstration of the polar capsule, silver nitrate impregnation method was used, by which the filament beginning at the anterior end is made visible (Fig. 614). The filament makes two or three turns in the space near the posterior end of the spore, where the sporoplasm is located. As the filament is coiled close to the wall of the polar capsule which is also close to the spore membrane, there is no place for annular sporoplasm situated outside the capsule. The biconcave thickening or ring form which is seen in mature microsporidian spores stained according to ordinary methods and which has been thought by numerous investigators to be the sporoplasm, is only the contracted substance composing the polar capsule so seen that the optical cross-sections of one or two turns of the filament were mistaken as two or four nuclei of the imaginary sporoplasm (Fig. 615). The true nuclei of the sporoplasm are always found in the posterior portion of the spore. They are made visible even by an ordinary method of staining such as iron hematoxylin, safranin, etc., when the preparation is deeply stained and differentiated very carefully and slowly. The nuclei were recognized and figured by several investigators of Microsporidia, although their true nature was not understood. For instance, Weissenberg noticed them in all the spores of *Glugea hertwigi*, but he explained them as metachromatic granules. The observation and subsequent interpretation of the structure of a microsporidian spore depend on the fixation and staining. The authors obtained a satisfactory demonstration of the spore of the present species by staining with picro-carmin (Bouin-Duboscq). The sporoplasm is very small (Fig. 616). Length of the spores is 8.5μ.

PLISTOPHORA INTESTINALIS Chatton 1907
[Figs. 617, 618]

1907	***Plistophora intesinalis***	**Chatton**	**1907 : 800-801**

Habitat: Epithelial cells of mid-gut of *Daphnia magna* and *D. pulex*. The infected host animals were found in the basin of reptiles in the Museum of Paris.

Locality: France (Paris).

Vegetative form: Schizont grows and its nucleus multiplies. Thus plasmodium (pansporoblast) is formed. The spores become differentiated in the latter (Fig. 617).

Spore: Pyriform (Fig. 618). A vacuole at obtuse end. When stained there becomes visible a deeply staining equatorial band which separates the above mentioned vacuole from another one, the polar capsule, situated at the other end. Although various reagents were used the filament was not seen extruded. Length 3μ, breadth 2μ.

PLISTOPHORA MIYAIRII Kudo

1909	*Nosema anomalum* (?)	Miyairi	1909 : 133-139

Habitat: Digestive tract in the region of cephalothorax of *Atyephira* sp. (crustacean). The infected region was white or reddish in color.

Locality: Nippon (Fukuoka).

Vegetative form: Multinucleate sporont (?) is rounded, 15 to 30μ in diameter, or elongated. Its cytoplasm is structureless and pale, and contains numerous refringent bodies. These latter are round (less than 3μ in diameter) or oval (reaching 6μ by 3μ).

Spore: Oval; one end is attenuated, the other rounded. At the latter end, an iodinophilous vacuole is present, being usually near one side. Iodine causes the extrusion of the polar filament from the attenuated pole. Length 9μ (rarely 13μ), breadth 7μ (small rounded ones rarely 6μ in diameter), length of polar filament about 90μ (some shorter, others 120μ long).

Remarks: There is no doubt about its microsporidiae nature. Because of the large vegetative form, it is placed here provisionally under a new name.

PLISTOPHORA HIPPOGLOSSOIDEOS Bosanquet 1910
[Figs. 619-622]

1910	*Plistophora hippoglossoideos*	Bosanquet	1910 : 434-438

Habitat: The fin-muscle of *Hippoglossoides limandoides.*

Locality: England (?).

Vegetative form: The microsporidian produces small whitish nodules, round or oval in shape and 1 to 2 mm. in diameter, lying in the muscular tissue. The nodules are made up of honey-combed masses of small cysts, most of which contained ripe spores. The cyst 20 to 25μ in diameter, lies in a small amount of structureless or fibrous reticulum, apparently derived from the host, among which remains of muscle fibers were here and there visible. A slight degree of cellular infiltration was seen at the edge of the nodules, but the muscle fibers in the vicinity seemed normal and unaffected.

Lying in the reticular substance between the cysts containing ripe spores were a certain number of others filled with small, rounded bodies with usually one nucleus, sometimes two, which appeared to be the final stage of the sporoblast, just before the formation of the spore membrane. The observations on the development are fragmental. In some instances, four fragments, each with a single vesicular nucleus, were seen within a single compartment or pansporoblast; in another there were seven fragments, one of which contained two nuclei and was apparently dividing. In other cases there were irregular masses of protoplasm containing many nuclei, or a single mass of this nature, representing the original sporonts.

In these multinucleated forms the majority of the nuclei were generally solid in appearance, but among them were often one or two vesicular nuclei, possibly representing the rudiments of developing spores.

Spore: Oval or pyriform (Fig. 622). A polar capsule is at the smaller end, though no details, not even its exact shape, could be made out, owing to the minuteness of the spore. Towards the broader end (in some cases there was no observable difference in the form of the two ends), there was often visible a clear, rounded space appearing like a vacuole, and in most instances there was in this a central dot. Between this and the polar capsule was a small mass of cytoplasm in which in very favorable specimens it was possible to detect two minute nuclei. Stained spores were about 3.5μ long by about 2μ broad.

PLISTOPHORA LABRORUM Le Danois 1910

[Fig. 611]

1910	*Plistophora labrorum*	Le Danois	1910 : 210-211

Habitat: The hypodermal muscle layer and muscles of the body cavity, and liver of *Crenilabrus melops*. One fish, 15 cm. long, showed a voluminous tumor. The right side of the body was so much deformed that the scales were scarcely imbricated one another. The diameter of the tumor was about 2 to 3 cm. By the weight of the tumor the fish was lying inclined toward the right side. The contents of the tumor were chalky-white. The liver had a chalky granular appearance instead of being red and smooth, and its size was slightly reduced.

Locality: France (Roscoff).

Vegetative form: Undescribed.

Spore: Oval (Fig. 611). A large and refringent vacuole is located at one end. The rest of the spore has a granular aspect and is easily stained. A polar capsule is made visible at the other end under the effect of reagents. Length 3μ, breadth 2μ.

Remarks: The author does not state the reason why the species is placed in this genus. The same host species is known to be parasitized by *Ichthyosporidium giganteum*, a haplosporidian, first discovered by Thélohan and lately studied by Swarczewsky (1914). The species is provisionally listed here where the author placed it.

PLISTOPHORA ELEGANS Auerbach 1910

[Fig. 612]

1910	*Plistophora elegans*	Auerbach	1910c : 441

Habitat: Ovarium of *Abramis brama* × *Leuciscus rutilus*.

Locality: Germany (Karlsruhe).

Vegetative form: Meronts multinucleated at a certain stage. Sporulation takes place only in the ova of the host. Meronts are, however, found abundantly in the connective tissue of the host. The young stages seem to be carried by the blood stream.

Spore: Highly elongated and narrow (Fig. 612). Shell thick. The polar capsule is very large with a distinctly visible coiled filament. The sporplasm surrounds the capsule and contains two nuclei in a mature spore. Macrospores 10μ long by about 4μ broad. Microspores not mentioned.

Remarks: Since the description of the vegetative form is inadequate, it is difficult to find the reason why the species is placed in the genus. It is listed here provisionally. Auerbach added that the species may be identical with *P. mirandellae.*

PLISTOPHORA LONGIFILIS Schuberg 1910
[Figs. 623-632, 760, 768; Textfig. B3]

1910	*Plistophora longifilis*	Schuberg	1910 : 401-434

Habitat: Testis of *Barbus fluviatilis.* One male fish was examined. The microsporidian formed clear whitish and rounded spots of variable size distending the surface of the organ (Fig. 760). No effect on the part of the host fish was seen responding the invasion of the parasite. The nucleus of the infected host cell, however, were conspicuously hypertrophied (Fig. 768).

Locality: Germany (Heidelberg; July).

Vegetative form: Fully grown pansporoblasts are somewhat spherical and measure 18 to 45μ in diameter (Fig. 623). They possess a thin, but distinct membrane which stains feebly. The number of spores formed in each pansporoblast is inconstant and numerous. In fact, in a single section of a pansporoblast 20 to 30 spores often may be observed. The size of the pansporoblast is not proportional to the number of spores which are produced in it. The maximum diameters of the pansporoblasts with microspores and with macrospores are 30μ and 45μ respectively. The sporoblast (Fig. 627) is spherical and contains a single nucleus. Its cytoplasm stains deeply blue with Giemsa showing a few vacuoles at one part. The sporoblast becomes elongated and a vacuole appears at one end opposite the end where the nucleus is located. The shell is formed. Later another vacuole appears at the other end. Thus the cytoplasm is gradually concentrated into a transverse zone. From these changes it seems probable that the cytoplasm is rich in fluid substance.

Spore: Two sizes (Figs. 629-631). Pyriform; one end narrower and the other broader. The spore membrane is moderately thick, and does not take stain very deeply. With Giemsa, it is stained violet or reddish. Aniline dyes such as acid-fuchsin, light green, and water blue, however, stain it deeply.

In the fresh state the spore shows only a pale ring-like structure in the middle portion. In Giemsa stained sections, the sporoplasm presents itself as a deep blue ring-like zone with single nucleus which is oval and stained bright red. No evidence of the presence of the polar capsule was found. The polar filament is coiled up (about 33 times by calculation from the author's figures) directly under the spore membrane. It can be well seen in deeply stained spores, in which about six windings in the posterior space and an obliquely directed line at the anterior end can be traced. While water, iodine solution, limewater, hydrochloric acid (10 per cent.) did not affect the polar filament, ammonia water (over night) caused filament extrusion from the side near the tip of the spore. The polar filament is very fine and extremely long. The empty spore membrane is somewhat broader and more regularly oval than the ordinary spore. The spore membrane is not composed of two valves (Textfig. B3). The sutural line mentioned by Thélohan for *Thelohania giardi* is probably a degeneration product caused by the digestion of the spore. Besides the nucleus there occur metachromatic granules located free in the vacuole or attached to the inner surface of the shell wall. Macrospores measure up to 12μ long by 6μ broad. Microspores measure 3μ long by 2μ broad. The extruded filament reaches 380 to 450μ or even up to 510μ in length.

PLISTOPHORA sp. Mercier 1908

[Figs. 633-638]

1908	*Plistophora* sp.	Mercier	1908a : 372-381

Habitat: Adipose tissue of *Blatta orientalis*. The infected insects are easily noticed by their distended abdomen. The infected tissue shows chalky-white color instead of its otherwise transparent appearance. Larvae are infected more frequently than adults, showing that the infection is probably fatal to the host. The microsporidian occurs in a state of diffuse infiltration. The nucleus of the infected host cell undergoes abnormal mitotic division. The latter is asymmetrical, multipolar or irregular. The normal cell with *Bacillus cuenoti*, however, divides amitotically.

Locality: France (Nancy?).

Vegetative form: The meronts when stained are small rounded bodies measuring 2 to 3μ in diameter and containing numerous chromatic granules (Fig. 633). After active multiplication, the latter increase in size and number. Each of the chromatic masses becomes surrounded by a small body of cytoplasm. The meront now becomes a sporont and then a pansporoblast. The number of spores formed in a pansporoblast varies but is always more than eight (Figs. 634, 635). When fully grown, the delicate membrane of the pansporoblast breaks up and the spores are set free in the host tissue.

Spore: Ovoidal. The polar capsule is distinctly visible (Fig. 637). The filament is extruded under the action of nitric acid (Fig. 638). Spore membrane is bivalve (Fig. 636). Fresh spores measure 5 to 6μ long by 2.5 to 3μ broad.

PLISTOPHORA sp. Drew 1909

1909	*Plistophora* sp.	Drew	1909 : 193-194

Habitat: The muscle of *Gadus* sp. A fish was examined. Diffused pigmented areas of brownish color were present in various parts of the muscular tissue. The boundaries of these areas were ill defined, but the pigmentation was more intense in the central portions, where the tissue showed marked degeneration. None of the viscera was examined. In sections, muscle fibrillae were partially or in places completely, replaced by a finely granular material contained within the sarcolemma: the shape of the fibrils was seldom altered, but sarcous elements within the sheath were usually destroyed. In the surrounding regions, some inflammation was present, and the interfibrillar space contained many leucocytes: the striation of the muscle fibers was also somewhat less pronounced than in the normal tissue. The "yellow bodies" were plentiful in the center of the lesions.

Locality: Off Iceland coast (January).

Vegetative form: Small spherical vesicles 30μ in average diameter are found in the inter- or intrafibrillar tissues. The membrane is thin and structureless, but resistant to potassium hydrate solution. Number of spores included is more than eight.

Spore: Oval. Polar capsule was not seen. The spore contains a small granulated area which is stained by hematoxylin and basic dyes and which probably represents the nucleus. Dimensions not given.

PLISTOPHORA DESTRUENS Delphy 1916

1916	*Plistophora destruens*	Delphy	1916 : 71-73

Habitat: The muscles of *Mugil auratus*. The posterior part of the host body was bent and curved in a remarkable way. The infected muscle fibers underwent degeneration.

Locality: France (Tatihou; April).

Vegetative form: Pansporoblasts at various stages of development were found in the host tissue. They were yellowish orange to yellow ochre and possessed a persistent membrane. When mature, they assumed a polygonal form.

Spore: Elongated ovoidal or pyriform. A polar capsul is peresent at the narrow extremity and a large aniodinophilous vacuole at the rounded end. In others very small granules which stain deeply with picrocarmin are seen at the latter end. Length 2.5 to 3.5μ, breadth 1.5 to 2.5μ, the filament 10 to 12 times longer than the spore.

Remarks: The generic characters are not mentioned by the author. It is placed here provisionally.

PLISTOPHORA SCIAENAE Johnston et Bancroft 1919

1919	*Plistophora sciaenae*	Johnston and Bancroft	1919 : 526-527

Habitat: The ovary of *Sciaena australis*. A single host fish was examined.

Locality: Australia (Ipswich).

Vegetative form: The microsporidian forms cysts. The infection apparently starts in the connective tissue covering the ovary, but as growth proceeds, the cyst becomes pressed down among the developing ova, though it is still surrounded by a hypertrophied layer of connective tissue.

Spore: Pyriform, with a mass of more deeply staining material at the narrow end. Length 3 to 5μ, breadth 2 to 3μ.

Remarks: The authors do not give a sufficient description of the stages of development, hence the generic designation is open to revision. As no reference is made to a polar filament, this form may not be a microsporidian. It is listed here provisionally.

Family COCCONEMIDAE Léger et Hesse 1922

The character of the family is described on page 67.

Genus COCCONEMA Léger et Hesse 1921

[Figs. 639, 640]

The character of the genus is stated on page 68.

Type species: *C. micrococcus* Léger et Hesse 1921

COCCONEMA MICROCOCCUS Léger et Hesse 1921

1921	*Cocconema micrococcus*	Léger and Hesse	1921 : 1420

Habitat: Adipose tissue of the larvae of *Tanypus setiger* (Diptera). The infected larvae appeared milky white and were swollen. They were sluggish in movements and died rapidly in the aquarium.

Locality France (Grenoble, Montessaux).

Spore: Diameter 1.8 to 2μ. Occasionally joined. Grouped in a spherical mass consisting of a large number of spores, or diffused throughout the tissue, giving to the observer the impression of a culture of micrococci.

COCCONEMA POLYSPORA Léger et Hesse 1921

1921	*Cocconema polyspora*	Léger and Hesse	1921 : 1420

Habitat: Adipose tissue of the larvae of *Tanypus* sp.

Locality: France (Grenoble).

Spore: Diameter 2 to 3.2μ. Grouped in a spherical mass containing a variable number (16, 32 or more) of spores.

COCCONEMA OCTOSPORA Léger et Hesse 1921

1921	*Cocconema octospora*	Léger and Hesse	1921 : 1420

Habitat: Epithelial cells of the intestine of the larvae of *Tanytarsus* sp. (Diptera).

Locality: France (Grenoble).

Spore: Diameter 2.1μ. Mostly eight spores are grouped together.

COCCONEMA SLAVINAE Léger et Hesse 1921

1921	*Coconnema slavinae*	Léger and Hesse	1921 : 1420

Habitat: Intestinal epithelium of *Slavina appendiculata*(Oligochaeta). The infected epithelial cells become hypertrophied.

Locality: France (Montessaux).

Vegetative form: Schizogony by amoeboid stages in the host cells.

Spore: Diameter 3μ. The spores are found in spherical or ovoidal masses containing a large number.

COCCONEMA STEMPELLI (Pérez 1905) Kudo
[Fig. 641]

1905	*Glugea stempelli*	Pérez	1905 : 15 1905c : 150-151

Habitat: Body cavity of *Balanus amaryllis.*

Locality: France (Persic Bay).

Vegetative form: White cysts spherical, have a diameter from one to two mm. Abundant in the region between the mantle and the shell, also in the place where the female glands are normally located. In section the cyst is surrounded by a thin membrane composed of the connective tissue of the host. The outer region contains numerous polymorphous and budding nuclei, the latter being about 20μ in length. These vegetative nuclei produce numerous small nuclei which migrate into the interior of the cyst. Each nucleus becomes surrounded by an island of cytoplasm and finally develops into a single spore. By this means the central portion of the cyst becomes filled with spores.

Spore: More or less spherical. Diameter about 1.5μ.

Remarks: Since the spore is spherical, the microsporidian is transferred from the genus Glugea into the genus Cocconema.

COCCONEMA MIYAIRII Kudo

1909	*Thelohania* sp.	Miyairii	1909 : 139-142

Habitat: The muscles of two species of *Atyephira*. The infected crustaceans showed a whitish or reddish white coloration of the body.

Locality: Nippon (Fukuoka).

Vegetative form: The sporont is tetrasporoblastic.

Spore: Spherical. Highly refractive. Various reagents failed to cause filament extrusion. Diameter 3μ.

Remarks: Although Miyairi's description is inadequate, the species is provisionally listed here under a new name, because of the spherical spores.

Family MRAZEKIDAE Léger et Hesse 1922

The characters of the family are described on page 68.

Genus MRAZEKIA Léger et Hesse 1916

The characters of the genus are described on page 68.

Type species: *M. argoisi* Léger et Hesse.

MRAZEKIA ARGOISI Léger et Hesse 1916

[Figs. 642-644; Textfig. B 5]

1916	*Mrazekia argoisi*	Léger and Hesse	1916 : 347

Habitat: In fat body surrounding the stomach of *Asellus aquaticus* (crustacean). Infected individuals died soon.

Locality: France (in the vicinity of Grenoble).

Vegetative form: Not specifically described. Same as in Nosema, a sporont developing into a single spore.

Spore: Tubular; without caudal prolongation. Some curved or slightly twisted, others ovoidal or pyriform. Length 17 to 23μ, breadth 3.5μ.

MRAZEKIA MRAZEKI (Hesse 1905)

[Figs. 652-655]

1905	*Myxocystis mrazeki*	Hesse	1905 : 914-916 1905b : 12-13

Habitat: *Limnodrilus hoffmeisteri*. In the epithelium and lumen of the intestine and body cavity of the annelid.

Locality: France.

Vegetative form: Generally spherical or ellipsoidal. Size up to 120μ in diameter. The form changes according to the pressure of the host tissue and also other parasites. Ectoplasm and endoplasm distinct. Ectoplasm very finely granular and homogeneous, often with immobile ciliary processes which disappear when the spores grow fully (Fig. 652). Endoplasm finely reticulated often contains vacuoles. Chromatic granules are found abundantly at the moment of sporulation. Ordinarily the endoplasm contains the nuclei and spores. Plasmotomy probably occurs. At the early stage the nuclei are of two kinds. One, smaller in number, is voluminous and spherical, having a chromatic membrane and fine chromatic granules scattered on the network. These nuclei remain until after sporulation. The other nuclei, smaller, irregular and exceedingly numerous, have also a membrane, one or two small karyosome and a network rich in chromatic substance. They multiply by mitosis. The centrosome is visible at the side of the nucleus. The above is a summary of Hesse's statements. According to the findings of Mrázek (1910) and Léger and Hesse (1916), the bodies described above are hypertrophied host cells.

Spore: Usually cylindrical with a small cylindrical process at the end (Fig. 654) from which the filament is extruded by immersing the spore in physiological solution for one or two hours (Fig. 655). Abnormal ovoidal, tubular, spherical or elliptical spores have been seen (Fig. 653). Some clearly showed the coiled filament. Length 9 to 10μ, breadth 1 to 2μ.

Remarks: Because of the form of the typical spore of the species, it is placed in this genus.

MRAZEKIA CAUDATA Léger et Hesse 1916

[Figs. 647-651]

1910	Myxocystis	Mrázek	1910 : 245-259
1916	*Mrazekia caudata*	Léger and Hesse	1916 : 347

Habitat: In the spermatocyte and lymphocyte of *Limnodrilus* sp. and in the lymphocyte of *Tubifex tubifex.*

Locality: Czechoslovakia and France (in the vicinity of Grenoble).

Vegetative form: Mrázek: The microsporidian attacks the spermatocyte and lymphocyte and causes extraordinary hypertrophy of the cells (Figs. 647-648). It is so small that the details cannot be studied. The membrane of the form found in Polamothrix was not uniform, one side being thicker than the other. The nucleus of the infected lymphocyte divides amitotically. Spore formation could not be studied; but it is clear that one schizont develops into a single spore.

Léger and Hesse: No special statement for the species. In agreement with Mrázek, the species is monosporous.

Spore: Léger and Hesse: Elongated and narrow cylinder (Fig. 651). A long caudal prolongation. Length of spore 16 to 18μ, breadth 1.3 to 1.4μ, caudal process about as long as the body of the spore.

MRAZEKIA BREVICAUDA Léger et Hesse 1916

[Fig. 645]

1916 *Mrazekia brevicauda* Léger and Hesse 1916 : 347

Habitat: Fat body of *Chironomous plumosus*. The infection was seen frequently.

Locality: France (in the vicinity of Grenoble).

Vegetative form: Same as that of *M. argoisi*.

Spore: Slender and elongated form with a short caudal prolongation which is hyaline, obtuse and narrower than the spore. Length 20 to 22μ breadth 1.4 to 1.5μ, caudal prolongation 3.5μ long.

MRAZEKIA STRICTA Léger et Hesse 1916

[Fig. 646]

1916 *Mrazekia stricta* Léger and Hesse 1916 : 347

Habitat: Lymphocytes of *Lumbriculus variegatus*. The infected lymphocytes reach a diameter of 100μ.

Locality: France (Dauphine).

Spore: Tubular, straight or sometimes slightly curved; destitute of caudal prolongation. Length 13 to 14μ, breadth 1.8 to 2μ.

MRAZEKIA TETRASPORA Léger et Hesse 1922

[Fig. 656]

1922 *Mrazekia tetraspora* Léger and Hesse 1922 : 327

Habitat: Adipose tissue of larvae of *Tanytarsus* sp. (Diptera).

Locality: France (Grenoble).

Vegetative form: A sporont forms four spores which are grouped in fours, but which become dispersed rapidly in the host tissue.

Spore: Cylindrical, straight or slightly curved. Length 6.5μ, breadth 0.8μ. A short hyaline prolongation at the posterior end is 1.2μ long.

MRAZEKIA BACILLIFORMIS Léger et Hesse 1922

[Fig. 657]

1922 *Mrazekia bacilliformis* Léger and Hesse 1922 : 327-328

Habitat: Adipose tissue of the larvae of *Orthocladius* sp. (Diptera).

Locality: France (Grenoble).

Vegetative form: Schizogony by uninucleated schizonts in chaplet form. A sporont forms eight spores which form a spherical group, but are cut up in rose-work. The spores eventually become scattered in the fat body and resemble bacilli.

Spore: Straight or very slightly curved; without caudal prolongation. Length 5μ, breadth 0.8μ.

Genus OCTOSPOREA Flu 1911 emend. Chatton et Krempf 1911

The characters of the genus are mentioned on page 68.
Type species: *O. muscae-domesticae* Flu 1911.

OCTOSPOREA MUSCAE-DOMESTICAE Flu 1911

[Figs. 658-663]

1911	*Octosporea muscae-domesticae*	Flu	1911 : 530-533
1911	*Octosporea muscae-domesticae*	Chatton and Krempf	1911 : 172-176, 179
1912	? Microsporidies	Cardamatis	1912 : 77-78

Habitat: Epithelial cells and muscles of mid-gut, hind-gut and collecting trunks of Malpighian tubules (larvae) and in the yolk of ovum of *Musca domestica* (Flu), *Drosophila confusa* and *D. plurilineata* (Chatton and Krempf). Chatton and Krempf note that the microsporidian probably has the ability of germinative infection of the next generation through the host ova. About 25 per cent. of the last named two hosts were infected; no infection occurred in *D. ampelophila*, *D. phalerata* and *D. funebris*. The microsporidian is particularly plentiful at the place where the Malpighian tubules are attached to the gut. Flu considered the organism a schizogregarine.

Locality: Dutch East Indies (Surinam), France (Paris).

Vegetative form: Chatton and Krempf: The young schizonts are found in the epithelial cells of the gut, measuring 3μ in diameter. Each possesses a single nucleus 2μ in diameter, with a centrally located karyosome and quite abundant peripheral chromatin grains. The nuclear division is mesomitotic. Schizogony seems to take place repeatedly. The schizont which becomes a sporont cannot be distinguished from an ordinary schizont. The nucleus of the schizont divides successively forming 8 or 16 or rarely 32 nuclei. When this is completed, there are ordinarily 8 or 16 binucleated bodies—the sporoblasts. The further development of each sporoblast is difficult to trace, but seems to proceed in general as follows: The two nuclei shift their positions and become located near the center of the body along the axis. At the moment there appear two nuclear grains (their origin unknown) which move to one of the extremities. Between them and the nuclei, a large vacuole becomes differentiated. At the opposite end of the sporoblast, there is formed a spherical body closely located to the distal nucleus, which increases in size, stains deeply and is visible even in a mature spore. This is the polar capsule. When the spore membrane is formed, the nuclei become diffused and connected with the polar capsule, thus giving the spore a typical appearance. The extruded filament was rarely seen (about 5 or 6μ long measured after the figure given by the authors).

Spore: Flu: Sickle shaped (Fig. 659). 8μ long.

Chatton and Krempf: Ovoidal or pyriform (Fig. 662, 663). Slightly attenuated at one end. Length 5 to 6μ, breadth 1μ. Highly refractive.

Remarks: Compare with *Thelohania ovata.*

OCTOSPOREA MONOSPORA Chatton et Krempf 1911

[Fig. 664]

1911	*Octosporea monospora*	Chatton and Krempf	1911 : 176-177, 179
1914	*Octosporea monospora*	Brug	1914 : 127-138

Habitat: Epithelial cells of the midgut of *Drosophila confusa* and *D. plurilineata* (Chatton and Krempf) and of *Homalomyia scalaris* (Brug).

Chatton and Krempf state that the microsporidian often produces large clusters which fall into the gut lumen. 90 per cent. of the laboratory bred flies were infected. Adults were more frequently infected than the larvae. There is, however, no direct continuity between the infections of the larvae and flies, since during the course of histolysis the spores, together with the host larval epithelial cells are cast off into the gut lumen and the spores find their way anew into the cells of the imago at the time of hatching. No parasites were found in the imaginal epithelium. Whether an autoinfection occurs or not is unknown.

Brug saw the microsporidian in all the Homalomyia larvae he studied in November, but in January and February less intensively, and the vegetative forms were not found any more. Although the spores were present in all the larvae, in many cases only a very few were seen.

Locality: France (Paris) and Holland (Amsterdam ?).

Vegetative form: Chatton and Krempf: The sporont is monosporous. The sporoblast is binucleated. Both uninucleated and binucleated bodies are found in the epithelial cells of the gut.

Brug: The schizonts are rounded membraneless cells. The cytoplasm stains more or less irregularly although it is not distinctly vacuolated. It has a compact nucleus, its periphery staining more deeply than the central portion. The uninucleated schizont measures 4 to 5μ in diameter. Schizogony is binary fission. The nucleus divides amitotically. Often multinucleated bodies are found. The final stage of schizogony is uninucleated oval body which directly develops into a sporont which in turn develops into a single spore.

Spore: Chatton and Krempf: Crescent in form. One extremity is slightly narrower than the other. Length 4 to 5μ, breadth 1μ.

Brug: Fresh spores are refractive and do not show any structure. At one end, there is a "polkörper" and at the other end a vacuole. The sporoplasm occupies the remaining portion and contains a single rod-shaped nucleus whose position is always definite. Various reagents failed to cause filament extrusion. The spore membrane is uniformly thick and there is no indication that it is composed of two shell-valves. Dimensions not given.

Remarks: Compare with *Thelohania ovata.*

Genus SPIRONEMA Léger et Hesse 1922

The characters of the genus are stated on page 68.

Type and only species: *S. octospora* Léger et Hesse 1922.

SPIRONEMA OCTOSPORA Léger et Hesse 1922

[Fig. 665]

1922	*Spironema octospora*	Léger et Hesse	1922 : 328

Habitat: Adipose tissue of the larvae of *Ceratopogon* sp. (Diptera).

Locality: France (Montessaux, Haute-Saône).

Vegetative form: The sporont is octosporous and often present in abundance in the hypertrophied host cells.

Spore: Spiral in form. Length 8 to 8.5μ, breadth 1μ. At the posterior extremity, one sees an oval vacuole, around which a spiral filament may be demonstrated by impregnation methods. The filament is 100μ long.

Genus TOXONEMA Léger et Hesse 1922

The characters of the genus are mentioned on page 68.

Type and only species: *T. vibrio* Léger et Hesse 1922.

TOXONEMA VIBRIO Léger et Hesse 1922

[Fig. 666]

1922	*Toxonema vibrio*	Léger and Hesse	1922 : 328

Habitat: Adipose tissue of the larvae of *Ceratopogon* sp. (Diptera).

Locality: France (Montessaux, Haute-Saône).

Spore: The spores are observed in immense number, diffused throughout the host tissue. In some pansporoblasts an octosporous character was noticed. Comma-shaped or in form of an arc of a circle. The direct distance between the two extremities is less than 2μ. Length of spore is about 3.5μ, breadth smaller than 0.3μ. At the slightly distended end, a vacuole is present. The two extremities do not seem to be in the same plane.

Suborder DICNIDEA Léger et Hesse 1922

The characters of the suborder are described on page 68.

Family TELOMYXIDAE Léger et Hesse 1910

Genus TELOMYXA Léger et Hesse 1910

The characters of the genus are mentioned on page 69.

Type and only species: *T. glugeiformis* Léger et Hesse 1910.

TELOMYXA GLUGEIFORMIS Léger et Hesse 1910

[Fig. 667]

1910	*Telomyxa glugeiformis*	Léger and Hesse	1910 : 413-414

Habitat: Fat body of the larvae of *Ephemera vulgata*. The infected larvae were chalky white and their movements very sluggish. The parasite was fatal to its hosts.

Locality: France.

Vegetative form: At the end of the development, the microsporidian completely occupies the host tissue, the host not reacting against the invasion of the parasite. The microsporidian was represented by numerous spores either free or in groups of 8, 16 or more, which showed that mictosporous sporulation probably occurs.

Spore: When examined in fresh conditions, the spore is refringent and does not show any structure. Its general appearance shows that it is a microsporidian spore. Ovoidal, but usually elliptical with equally rounded extremities. Length 6.5μ, breadth 4μ. When fixed and stained, each spore exhibits two polar capsules which are located end to end and fill completely the equatorial zone. Around the point of contact of the capsules and in the extracapsular cavity, the binucleated sporoplasm is to be seen. Further one sees two nuclei for shell valves and two for the polar capsules. In each polar capsule, a long slender filament of about 90μ in length is present. The latter is often extruded laterally.

AMBIGUOUS FORMS

In the literature one finds numerous references to Nosema, Glugea or Microsporidia, the real nature of which cannot be determined since the observations and the descriptions are mostly inadequate. Some of the forms which had formerly been called Microsporidia, have been proven by more recent investigators to belong to other groups of Neosporidia, particularly to the Haplosporidia. Prominent among them are *Ichthyosporidium* (*Glugea*) *giganteum* (Thélohan) Swarczewsky and *Coelosporidium periplanetae* (Lutz et Splendore) Swarczewsky. Thélohan (1895) found the first named form in *Crenilabrus melops* and held it to be a microsporidian, giving the name *Glugea gigantea*. Swellengrebel (1912) studied its life history and placed it in the genus Plistophora. Both authors failed to cause filament extrusion of fresh spores by using various reagents which were efficient in bringing the filament out from a typical microsporidian spore.

Lutz and Splendore (1903) observed a microsporidian-like organism in the Malpighian tubules of *Periplaneta americana* and named it *Nosema periplanetae*; it had been mentioned by Schaudinn (1902) as a Nosema. This form was studied further by Perrin (1906), Shiwago (1909), Epstein

(1911) in *Blatta orientalis* and *Blatella germanica.* These latter investigators also held it to be a microsporidian and placed it in the genus Plistophora, although the polar filament was not observed. Crawley (1905) probably studied this form and considered it a haplosporidian, naming it *Coelosporidium blattellae.* Swarczewsky (1914) came to the conclusion that the two last mentioned forms are in reality Haplosporidia. Under these circumstances, it will not be at all surprising to find in future that many of the ambiguous forms listed here when studied again will be placed in orders other than Microsporidia.

Gen. et spec. incert. (Frey et Lebert)

1856		Frey and Lebert	1856 : after Pfeiffer
1858		Lebert	1858 : 161

Habitat: In *Ocypus* (*Emus*) *olens* (Coleoptera).
Locality: Switzerland.

Gen. et spec. incert. (Leydig)

1855		Leydig	1855 : 397
1863		Leydig	1863 : 187
1895	*Glugea* sp.	Pfeiffer	1895 : 39

Habitat: The muscles of heart and trunk of *Aranea* (*Epeira*) *diadema* (Arachnida).
Locality: Germany.
Spore: Oval. Refringent. Length 4μ.

Gen. et spec. incert. (Leydig)

1863		Leydig after Pfeiffer	
1895	*Glugea* sp.	Pfeiffer	1895 : 39

Habitat: The muscle of *Apis mellifica.*
Locality: Germany.

Gen. et spec. incert. (Vlacovich)

1867		Vlacovich after Pfeiffer	
1895	*Glugea* sp.	Pfeiffer	1895 : 39

Habitat: In *Gryllus campestris* (Orthoptera).
Locality: Italy.

Gen. et spec. incert. (Balbiani)

1882		Balbiani	1882 : 1168-1171
1899	*Nosema* sp.	Labbé	1899 : 107

Habitat: The larvae of *Antherea* (*Attacus*) *pernyi* and *A. yamamai* (Lepidoptera).

Locality: France.

Remarks: Compare with *Nosema* sp. Ishiwata.

Gen. et spec. incert. (Balbiani)

1882		Balbiani	1882 : 1168-1171
1899	*Nosema* sp.	Labbé	1899 : 107

Habitat: *Platycleis grisea* (*Decticus griseus*) (Orthoptera).

Locality: France.

Gen. et spec. incert. (Frenzel)

1892		Frenzel	1892 : 283-285
1899	*Nosema* sp.	Labbé	1899 : 108

Habitat: The Malpighian tubules of *Statira unicolor* (Coleoptera). Frenzel observed that the tubules were filled irregularly with the parasites.

Spore: The form of the parasite was same both in free and encapsuled conditions. A thick bacilliform type with somewhat pointed ends or hemispherical. Some are straight, others are bean-shaped. The shell ("cuticula") is highly refractive and the protoplasm homogeneously opaque. Vacuole-like structures are seen. Treatment with iodine alone or in combination with acetic acid and sulphuric acid, brings a light yellow coloration of the shell and the protoplasm. It is not attacked by strong nitric acid nor acetic acid, while concentrated sulphuric acid destroys it rapidly. Length 12 to 13μ, breadth 3.5μ.

Gen. et spec. incert. (Frenzel)

1895		Frenzel	1895 : 272-274
1899	*Nosema* sp.	Labbé	1899 : 108

Habitat: Epithelial cells of mid-gut of *Porthesia chrysorrhoea* (Lepidoptera). Frenzel described the form as an enclosure (?) in the epithelium. Later he referred to it as a parasite.

In the larvae the epithelial cells of the mid-gut were found to contain spherical bodies which were about 12μ in diameter with a distinct membrane, and contained a number of cylindrical bodies (spores ?). In the adult the epithelial cells with such bodies were more abundant than in the larva, and the number of the bodies in a host cell was far greater, in some cases reaching 1000. Under the microscope, the field was often full of these structures due to the rupture of the cell membrane.

Locality: Germany.

Spore: Cylindrical with hemispherical ends; slightly curved. Colorless and highly refractive. It is insoluble in cold and warm acetic acid, but disappears in concentrated sulphuric acid and slowly in hydrochloric acid. Nitric acid, ammonia, caustic-potash, alcohol or solvents of fat caused no changes. Length 8μ, breadth about 2.5μ.

Gen. et spec. incert. (Müller)

1894		Müller	1894 : 18
1899		Labbé	1899 : 110

Habitat: In the shell and body of *Paradoxostoma* sp. (crustacean).

Locality: Italy (Naples).

Gen. et spec. incert. (Pfeiffer)

1895	*Glugea* sp.	Pfeiffer	1895 : 52

Habitat: In the Malpighian tubules of *Melasoma* (*Chrysomela*) *populi* (Coleoptera). Two infected animals were studied.

Locality: Germany (Weimar).

Gen. et spec. incert. (Pfeiffer)

1895	*Glugea* sp.	Pfeiffer	1895 : 39

Habitat: In *Vespa media*.

Locality: Germany.

Gen. et spec. incert. (Pfeiffer)

1895	Glugea	Pfeiffer	1895 : 52

Habitat: The ovary, ovum and fat body of the larvae of *Potamanthus* sp. (?), ephemerid. The infection was rare, three infected larvae being found.

Locality: Germany (Tiefurt and Hetschburg).

Vegetative form: The pansporoblast contained 8, 18 or more spores.

Spore: Pyriform with a large vacuole at the broad end.

Gen. et spec. incert. (Fritsch)

[Fig. 668]

1895	Glugea	Fritsch	1895 : 81

Habitat: The posterior part of the abdomen of *Daphnia kahlbergensis* and *Ceriodaphnia quadrangula*. The infection was rare.

Locality: Hungary (Unter-Pocernitzer Teiche).

Vegetative form: Oval cyst of 30μ in diameter.

Spore: Not mentioned. Fritsch's figures probably represent spores. If so, they are pyriform with or without a vacuole at the rounded end.

Gen. et spec. incert. (Fritsch)

1895 Glugea? Fritsch 1895 : 85

Habitat: *Actinurus neptunius* (Rotifera). The parasite was found often.

Fritsch found granular bodies in the body of the host, which corresponded in their form to spermatozoa of the host, but since no male nor hermaphrodite is known in the host species, he supposed them to be parasitic forms.

Gen. et spec. incert. (Pfeiffer)

1895 Glugea Pfeiffer 1895 : 38

Habitat: *Leuciscus phoxinus* (*Phoxinus laevis*) (Pisces).
Locality: Germany (G. W. Müller 1894, after (Pfeiffer).

Gen. et spec. incert. (Kulagin)

1898 *Glugea bombycis* Kulagin 1898 : 469-471

Habitat: Silk-glands, Malpighian tubules, muscles of the mouth part, and other organs of the larvae of *Lyda nemoralis*. 70 to 80 per cent. of the larvae examined were infected. The infected larvae move about sluggishly and lose their appetite. On the skin especially of the dorsal side of the body, numerous irregularly contoured dark brownish spots were observed; they were not seen at all on healthy larvae.

Locality: Russia.

Vegetative form: Two kinds were noticed. One, a protoplasmic sac with mature spores of oval shape and uniform size, frequently observed in the silk glands and fat body. It resembles the figure given by Balbiani for *Nosema bombycis*, but differs from the latter in that it has larger amount of protoplasm and more massive nucleus of spherical form. The other, a second sac contained spores of two kinds and was found less frequently and exclusively in fat body. These spores were spherical with distinct envelope and nucleus, one being twice as big as the other. Kulagin considered these two different bodies to have same significance as the microgametes and macrogametes of other Sporozoa.

Spore: Form, structure and size resemble strikingly those of *N. bombycis*. Oval. Shell is thicker and more sharply defined than that of the latter. Sometimes, forms three or four times larger than the normal and of very variable shape such as pyriform, reniform, irregularly triangular, etc., were found. Staining with Heidenhain reveals that the vacuole seen in fresh state is none other than the nucleus. Length 6 to 7.2μ, breadth 5 to 7μ.

Gen. et spec. incert. (Christophers)

[Figs. 669, 670]

1901	Sporozoan	Christophers	1901 : 20
1921	Sporozoan	Nicholson	1921 : 441-442
1924	*Thelohania* sp. ?	Kudo	1924a (in press)

Habitat: Ova of *Anopheles* sp. (Christophers) and *A. maculipennis* (Nicholson). Frequent occurrence.

Locality: England.

Christophers: Frequently in Anopheles a large portion or the whole of the adult ovum consists of a mass of Sporozoa. These consist of numerous small cysts, each containing eight round or crescent-shaped bodies, each with a central chromatin spot (Fig. 669).

Nicholson: As has already been observed by S. R. Christophers, the yolk of a mosquito egg is frequently entirely displaced by a mass of Sporozoa. These appear as transparent spherical cysts 5μ in diameter, approximating in size to the coarse yolk granules; in them are found eight small bodies which take up stain. In sections this number is not constant, but there are never more and the reduced number is probably due to the removal of part of the cyst (Fig. 670). This is the only stage of the organism which the author observed and, though a number of insects were found affected, the cysts were only observed in mature oocysts.

Gen. et spec. incert. (Linton)

[Fig. 671]

1901	Protozoan	Linton	1901 : 433

Habitat: In the intestine (contents) of *Dasyatis* (*Trygon*) *centrura* (Pisces).

Locality The United States (Woods Hole; July).

Linton: Enormous numbers of small bodies were seen, long elliptical in outline and measuring 14μ long by 6μ broad.

Gen. et spec. incert. (Linton)

1901	Protozoan	Linton	1901 : 455

Habitat The liver of *Rhombus* (*Stromateus*) *triacanthus*.

Locality: The United States (Woods Hole; September).

Vegetative form: Sporocysts are white, globular and measure 1.5 mm. in diameter. When compressed they liberated immense number of spores which were in large part aggregated into globular or oblong clusters, the larger as much as 0.02 mm. in diameter.

Spore: Short and thick; with bluntly rounded ends. Length about 2.5μ, and a little less than that in breadth and thickness.

Gen. et spec. incert. (Grassi)

[Fig. 672]

1901	Sporozoan	Grassi	1901 : 448
1922	*Glugea* (?) sp.	Kudo	1922 : 70, 72

Habitat: Body cavity, intestine, salivary glands and dorsal vessel of *Anopheles* sp.

Locality: Italy.

Grassi: In a probable young stage of the free form, it is a rounded multinucleated protoplasmic mass. It contains oval bodies, each with a brilliant central corpuscle and capable of escaping from the mass. When attached to the host organs which condition is more commonly found than the free state, it appears tubular or irregularly globular. It becomes surrounded by a membrane and the contents break up into a large number of spores each provided with an envelope. The polar capsule was not seen.

Gen. et spec. incert. (Grassi)

[Fig. 673]

1901	Sporozoan	Grassi	1901 : 449
1922	*Thelohania* (?) sp.	Kudo	1922 : 70, 72

Habitat: Ovum of *Anopheles* sp.

Locality: Italy.

Grassi: In the protoplasmic mass one finds two, four or eight nuclei. A capsule is formed when eight sporozoites are produced in the mass.

Gen. et spec. incert. (Ross)

1906	Protozoan	Ross	1906 : 104
1922	*Thelohania* (?) sp.	Kudo	1922 : 71, 72

Habitat: In the nerve chord of imago of *Stegomyia* sp. and *Culex fatigans*.

Locality: India.

Vegetative form: Exactly eight spores were closely packed within an oval envelope.

Spore: Oval. Refractive. Apparently hard. A circular vacuole was seen at one focus of the ellipse.

Gen. et spec. incert. (Stephens et Christophers)

1908	Nosema (?)	Stephens and Christophers	1908 : 115
1921	Nosema (?)	Kudo	1921a : 155

Habitat: Oesophageal diverticula of *Culex* sp.

Locality: England.

Gen. et spec. incert. (Keysselitz)

1908	Microsporidium	Keysselitz	1908 : 276

Habitat: Ovary and ovum of *Barbus fluviatilis*. The author observed in a single fish a parasite that apparently belong to the Microsporidia. This may possibly be identical with *Plistopora longifilis*.

Locality: Germany (Neckar).

Gen. et spec. incert. (Guyénot et Naville)

1922	Microsporidie	Guyénot and Naville	1922 : 419-421

Habitat: Enclosed in a connective tissue cell of *Rana temporaria* found in the ectoplasm of *Myxobolus ranae*, a myxosporidian, parasitic in *Rana temporaria*. The authors think that migration of a host cell into the trophozoite of the myxosporidian took place accidentally.

Spore: Ovoidal. Length 4μ.

Gen. et spec. incert. (Guyénot, Naville et Ponse)

1922	Microspridie	Guyénot, Naville and Ponse	1922 : 635-636

Habitat: In a cestode which seems to be closely related to *Ligula colubri blumenbachii* Cobbold, parasitic in *Tropidonotus natrix*.

Locality: Italy.

Spore: Length 2 to 2.5μ, breadth 1.5μ.

Genus et species incert. (White)

1923	Neosporidian	White	1923 : 359

Habitat: The fat body of *Tribolium confusum* and *T. ferrugieneum*. The parasite is found very largely in the host cells. The infection often does not extend throughout the fat body. The larvae may show symptoms of the disease in a migration of some of them from the food (ground wheat, corn, and other cereals) to the sides of the jar and to the cotton plug. Positive signs of infection in the individual insect are often difficult to detect. Late in the course of the disease the sick larva may be less active, slightly distended, whitish, and show increased opacity. The larvae die usually while they are in the food, although occasionally the remains are found in the cotton of the plug. Death occurs not only during the larval period, but during the pupal and adult stages also. As a rule a general infection of the insect culture is not noted for more than a month after inoculation. After a few months most of the insects are dead of the disease.

Locality: The United States (Washington).

Vegetative form or spore: Not described.

Gen. incert. *helminthophthorum* (Keferstein)

1862	*Mucor helminthophthorus*	Keferstein	1862 : 135
1887	*Nosema helminthorum*	Moniez	1887a : 1312
1899	*Plistophora helminthophthora*	Labbé	1899 : 111

Habitat: Parenchyma and genital organs of *Taenia expansa, T. denticulata, T. bacillaris* and *Ascaris mystax.*

Locality: Germany and France.

Vegetative form: Moniez: The spores are found in an enormous number in the host tissue. They enter the ovary and retard the development of the organ and further infect new hosts.

Labbé: Sporogeneous masses are spherical and measure 20μ in diameter.

Spore: Moniez: Oval. Length 5μ, breadth 2.5μ. Optical characters and resistance against chemical reagents are similar to those of *Nosema bombycis.*

Labbé: Oval and refringent. Length 4.2 to 5.9μ, breadth 1.7 to 2.5μ.

Gen. incert. *strictum* (Moniez)

1887	*Nosema stricta*	Moniez	1887a : 1313

Habitat: Connective tissue, muscle and fat body of *Pachyrhina pratensis* (Diptera) and *Zygaena filipendulae* (Lepidoptera).

Locality: France.

Gen. incert. *heteroica* (Moniez)

1867		Vlacovich	1867 : 5
1887	*Nosema heteroica*	Moniez	1887a : 1313

Habitat: In *Zamenis gemonensis* (*Coluber carbonarius*) (reptile).

Locality: Italy.

Vegetative form: Spherical vesicle forms 8, 16, 64 or more spores. Each sporoblast (?) is 12 to 18μ in diameter.

Spore: Ovoidal, with a clear vacuole at one end. Length 6 to 7μ, breadth 2 to 3μ.

Genus incert. *schmeilii* (Pfeiffer)

[Fig. 674]

1890		Schmeil	1890 : 19-21
1895	*Glugea schmeilii*	Pfeiffer	1895 : 84-86, 119 1895a : 61-63, 72

Habitat: *Cyclops* sp. *Diaptomus vulgaris* (*D. coeruleus*), *D. salinus* (*D. richardii*). After Pfeiffer Schmeil mentioned that the infected animal could be distinguished from the normal one by the grey color of the body. Movements of the host were normal. Upon microscopical examination, the dark parasitic masses are seen in the anterior part of the body, abdomen, first antenna and swimmerets. He in vain tried to infect the normal host animal with the parasite.

Locality: Germany (Halle, Heidelberg, Greifswald, Ludwigshafen).

Spore: Spindle to half-moon form. Size variable, though uniform in one and the same host. The spores were unaffected in glycerine or water. Pfeiffer: Length 4 to 8μ, breadth 3 to 6μ.

Gen. incert. *holopedii* (Fritsch et Vavra)

[Figs. 675, 676]

1894	*Microsporidium holopedii*	Fritsch and Vavra	1894 : 106
1895	*Glugea leydigii* (part.)	Pfeiffer	1895 : 65, 72
1895	*Glugea holopedii*	Fritsch	1895 : 79, 80

Habitat: In *Holopedium gibberum* (crustacean). Fritsch observed 8 to 33 per cent. of infection. The parasite appears as white masses.

Locality: Hungary (July, August).

Vegetative form: Fritsch observed the cysts (pansporoblast ?) contained mostly 10 spores.

Spore: Fritsch: Pyriform with a distinct vacuole at one end. Under higher magnification, one sees cytoplasm, vacuole and nucleus in the rounded cyst (spore ?). Dimensions are not given.

Gen. incert. *colorata* (Fritsch)

[Figs. 677, 754]

1895	*Glugea colorata*	Fritsch	1895 : 80-81

Habitat: In *Diaptomus gracilis.*

Locality: Hungary.

Vegetative form: The parasite formed olive green masses. The cyst contained only five spores(?). In another host the color of the parasite was burnt sienna and each cyst contained six spores.

Spore Two forms were noted. Dimensions not given.

Gen. incert. *polygona* (Fritsch)

1895	*Glugea* (?) *polygona*	Fritsch	1895 : 85

Habitat: In *Asplanchna* sp.

Locality: Hungary (July).

Spore: Rounded hexagonal, grouped in 3, 6 to 13. A single nucleus is seen under higher magnification.

Gen. incert. *thysanurae* (Pfeiffer)

1895	*Glugea thysanurae*	Pfeiffer	1895 : 53-54, 73

Habitat: The reproductive organ of *Podula aquatica*.

Locality: Germany (February).

Spore: Spore has a small knob at one end, and shows a longitudinal line on the shell. Calculated dimensions: length 16μ, breadth 7μ.

Remarks: The author thought that there would be about two or three Glugea parasitic in the insect. In those caught on the snow in February at Wolfsberg, there were seen spores without the above mentioned knob. It is strange to note that Pfeiffer did not see any structure in the spore although the latter is of such unusual dimensions. Hence its microsporidian nature is doubtful.

Gen. incert. *coccoidea* (Pfeiffer)

1895	*Glugea cladocera* II	Pfeiffer	1895 : 66,
1895	*Glugea coccoidea*	Pfeiffer	1895 : 73
1899	*Plistophora coccoidea*	Labbé	1899 : 109

Habitat: The hypodermal cell of *Limnetis* sp. and *Daphnia pulex*.

Locality: Germany (Greifswald, Heidelberg, etc.).

Vegetative form: The sporoblasts, 2.5 to 4μ in diameter, contain 8, 16 or more spores.

Spore: Form resembles that of *Staphylococcus aureus* with a granular nucleus. Length 2 to 3μ.

Gen. incert. *rosea* (Fritsch)

1895	*Glugea rosea*	Fritsch	1895 : 81

Habitat: *Cyclops strenuus*. A single rose-colored host was observed.

Locality: Hungary.

Spore Two forms. One somewhat attenuated at one end, smaller and yellowish in color, the other elongated pyriform and larger with or without vacuole.

Gen. incert. *asplanchnae* (Fritsch)

1895	*Glugea* (?) *asplanchnae*	Fritsch	1894 : 83-84

Habitat: *Asplanchna* sp. The parasite appeared as large white masses

Locality: Hungary.

Spore: Oval; size variable.

Gen. incert. *geophili* (Crawley)

[Fig. 678]

1903	*Nosema geophili*	Crawley	1903 : 337-338

Habitat: Intestine of *Geophilus* sp. (Myriapoda). The smear of the intestine contained 30 to 40 specimens of the parasite, besides these were found innumerable individuals of the vegetative stage of a coccidian, probably a species of Eimeria.

Locality The United States (Cambridge, Mass.; May).

Vegetative form: Oval, with occasionally a blunt prolongation at one end. The cytoplasm is not differentiated. Smaller forms are mostly uninucleate. Larger specimens with numerous nuclei which were arranged in pairs, contained much less dense cytoplasm than the smaller forms. Size varied 30μ to 150 or 200μ. The nuclei were ellipsoidal and contained a large karyosome.

Spore: Not observed.

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PLATE I

EXPLANATION OF PLATE I

Figs. 1-39. *Nosema bombycis*. 1-20 after Stempell ×2250, 21-39 after Kudo ×2850 except 39 which is about ×2000.

Figs. 1 and 2. Planonts.

Fig. 3. A young meront from *Arctia caja*.

Fig. 4 to 7. Meronts in binary fission.

Figs. 8 to 14. Meronts in multiple division.

Figs. 15 and 16. Stained spores.

Fig. 17. A fresh spore.

Fig. 18. A young spore.

Fig. 19. A mature spore after being treated with nitric acid and studied with obliquely projected light.

Fig. 20. An abnormal spore (fresh) from *Arctia caja*.

Fig. 21. Young amoebulae.

Fig. 22. A young schizont.

Figs. 23 to 28. Schizogonic stages.

Fig. 29. A young sporont.

Figs. 30 and 31. Young spores.

Figs. 32 and 33. Mature spores.

Fig. 34. A spore treated with formol for 10 minutes and pressed mechanically under the cover glass.

Figs. 35 and 36. Spores treated with concentrated nitric acid.

Figs. 37 and 38. Spores kept in the digestive fluid of the host and studied after 24 hours.

Fig. 39. A spore with the extruded filament under the action of mechanical pressure.

Figs. 40 to 46. *Nosema bryozoides*. 40-42 after Korotneff; 43-46 after Braem.

Fig. 40. Part of an infected faniculus. ×350.

Fig. 41. An infected host cell ("creeping myxosporidium with nuclei and spores") ×750.

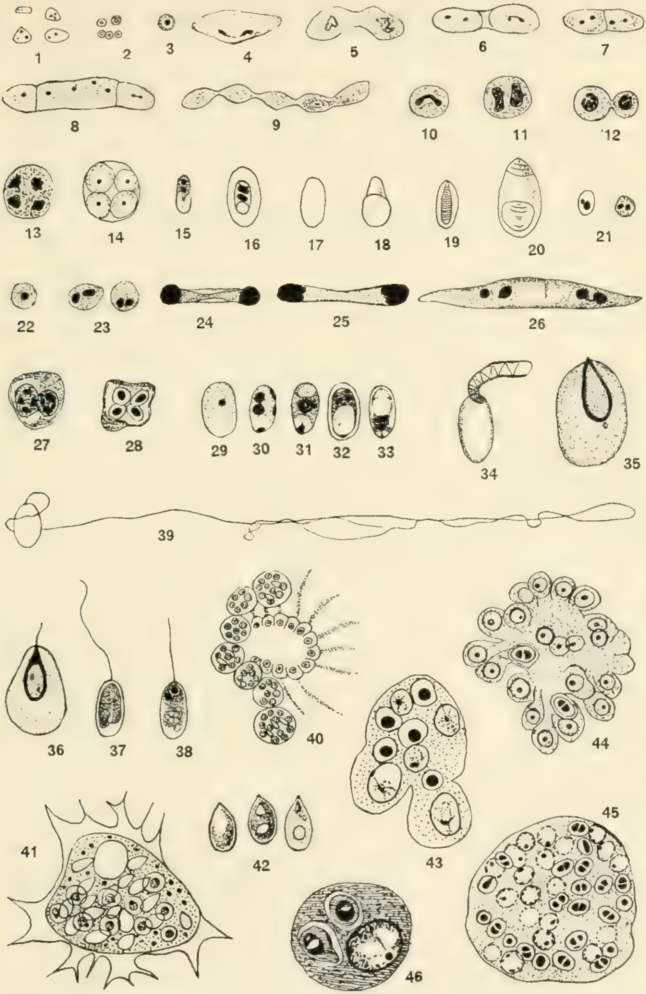
Fig. 42. Spores. ×900.

Fig. 43. Spermatoblasts developing into a plasmodium. ×900.

Fig. 44. Schizonts from the youngest part of the testis near body wall. ×600.

Fig. 45. A more advanced stage.

Fig 46. A testicular cell harboring two parasites. ×1100.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KUDO A STUDY OF THE MICROSPORIDIA PLATE I

PLATE II

EXPLANATION OF PLATE II

Figs. 47 to 60. *Nosema bryozoides*. 47-57 after Braem, 58-60 after Schröder.

Fig. 47. A part of an infected faniculus cut longitudinally, showing more developed portion of the testis. ×400.

Fig. 48. A nucleus of the testis cell from the last figure.

Figs. 49 to 56. Various division stages of the nucleus.

Fig. 57. A free "myxosporidium"-stage from the body cavity. ×600.

Fig. 58. A sausage form composed of numerous spermatogonia infected by the microsporidian.

Figs. 59 and 60. Two spores.

Figs. 61 to 63. *Nosema ciliata*. After Mràzek.

Fig. 61. A cross-section of *Limnodrilus claparedianus*, showing the parasitic masses.

Fig. 62. A floating form in the host's body cavity.

Fig. 63. A spore.

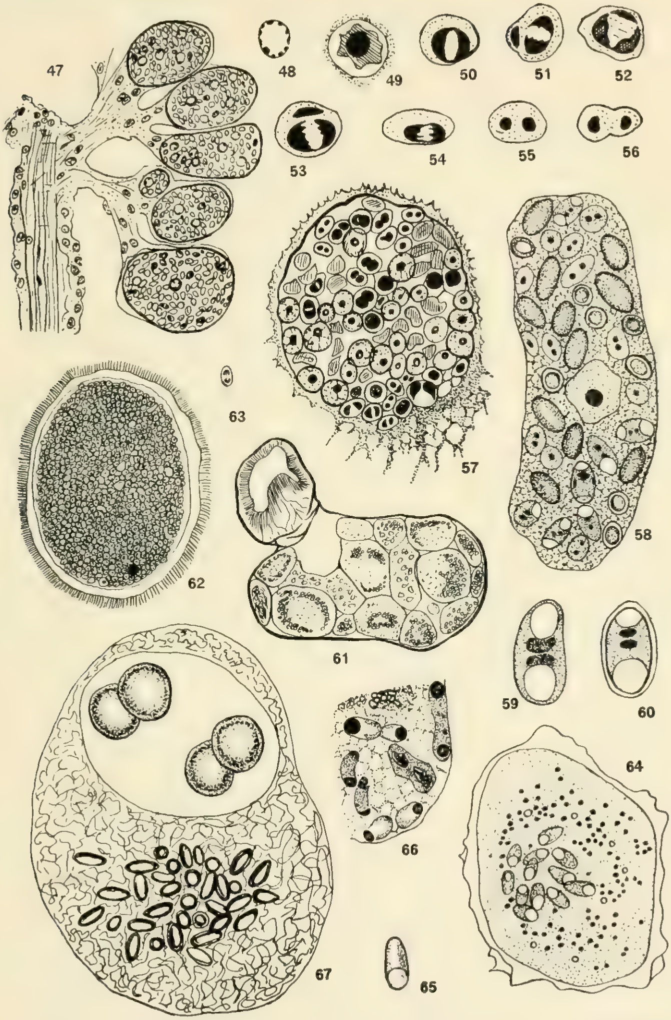
Figs. 64 to 67. *Nosema marionis*. 64, 65 after Thélohan; 66, 67 after Stempell. ×1900.

Fig. 64. A young trophozoite of *Ceratomyxa coris* infected by the microsporidian. ×750.

Fig. 65. A fresh spore. ×1500.

Fig. 66. A part of a host trophozoite, containing developmental stages of the microsporidian.

Fig. 67. A sporulating host trophozoite infected by the microsporidian.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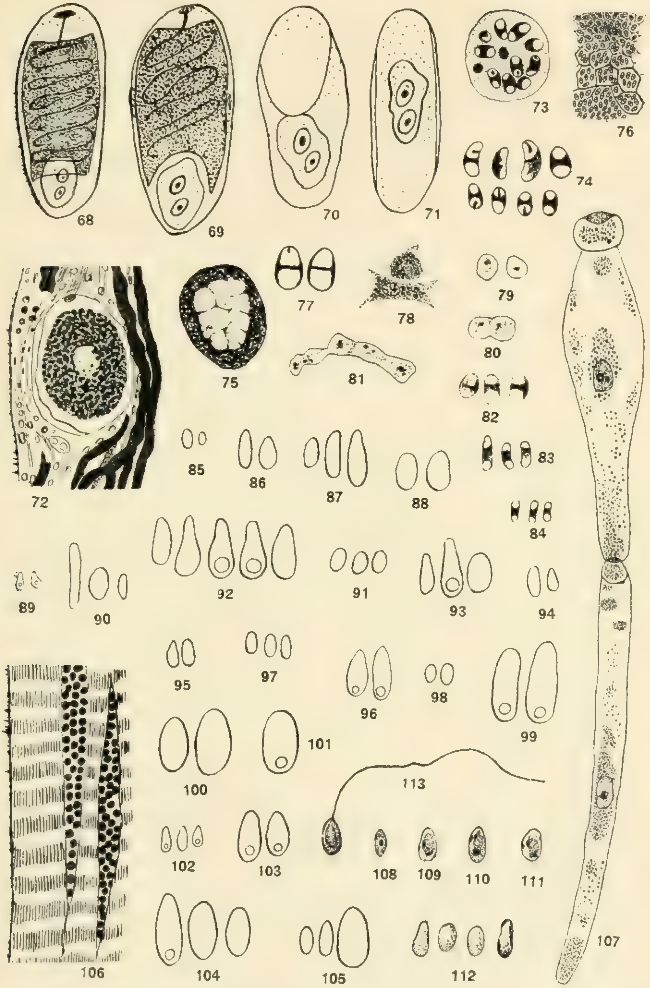
KUDO A STUDY OF THE MICROSPORIDIA PLATE II

PLATE III

EXPLANATION OF PLATE III

Figs. 68 to 71. Spores of *Nosema marionis* after Georgévitch.
Figs. 72 to 84. *Nosema lophii.* 72-74 after Doflein; 75-78 after Mrázek; 79-84 after Weissenberg.
Fig. 72. A section through infected host nerve fibers with one cyst.
Fig. 73. A pansporoblast (according to Weissenberg, a host cell).
Fig. 74. Eight stained spores.
Fig. 75. Cross-section of a cyst.
Fig. 76. A portion of the section of a cyst.
Fig. 77. Spores.
Fig. 78. Two phagocytes with numerous spores.
Fig. 79. Schizonts.
Figs. 80 and 81. Schizonts in the process of division.
Fig. 82. Stained oval spores.
Fig. 83. Stained larger cylindrical spores.
Fig. 84. Stained smaller cylindrical spores.
Figs. 85 to 105. After Lutz and Splendore.
Fig. 85. Spores of *Nosema vanillae α.* ×2000.
Fig. 86. Spores of *Nosema vanillae β.* ×2000.
Fig. 87. Spores of *Nosema vanillae γ.* ×2000.
Fig. 88. Spores of *Nosema astyrae.* ×2000.
Fig. 89. Spores of *Nosema girardini.* ×2000.
Fig. 90. Spores of *Nosema junonis α.* ×2000.
Fig. 91. Spores of *Nosema junonis β.* ×1250.
Fig. 92. Spores of *Nosema lysimniae.* ×2000.
Fig. 93. Spores of *Nosema eubules.* ×2000.
Fig. 94. Spores of *Nosema lophocampae.* ×2000.
Fig. 95. Spores of *Nosema erippi.* ×2000.
Fig. 96. Spores of *Nosema cacculiae.* ×1250.
Fig. 97. Spores of *Nosema hydriae* ×1295.
Fig. 98. Spores of *Nosema micrattaci.* ×1250.
Fig. 99. Spores of *Nosema sabaunae.*
Fig. 100. Spores of *Nosema auriflammae.*
Fig. 101. Spores of *Nosema mystacis.*
Fig. 102. Spores of *Nosema distomi.*
Fig. 103. Spores of *Nosema chironomi.*
Fig. 104. Spores of *Nosema ephialtis.*
Fig. 105. Spores of *Nosema balantidii.*
Fig. 106. A part of a section of the muscles of *Carcinus maenas* infected by *Nosema pulvis.* After Pérez. ×2000.
Figs. 107 to 113. *Nosema frenzelinae.* After Léger and Duboscq
Fig. 107. An associated couple of the host gregarine, *Frenzelina conformis* infected by the microsporidian.
Fig. 108. A schizont.
Figs. 109 to 111. Stained spores.
Fig. 112. Fresh spores.
Fig. 113. A spore with the extruded polar filament.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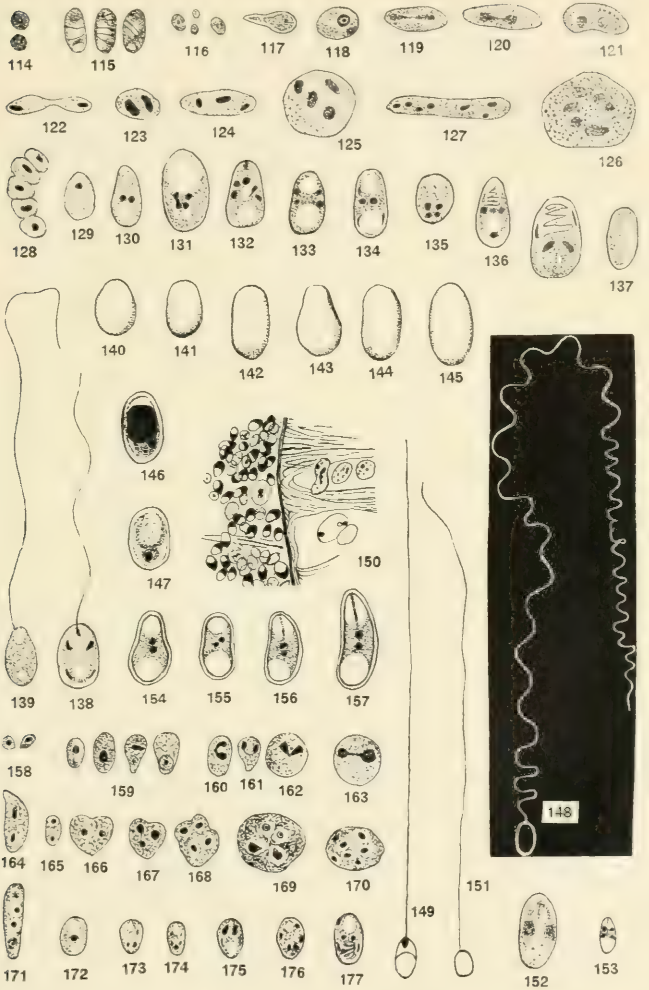
KUDO A STUDY OF THE MICROSPORIDIA PLATE III

PLATE IV

EXPLANATION OF PLATE IV

Figs. 114 to 148. *Nosema apis.* 114, 115 after Zander; 116-139 after Fantham and Porter ×1500; 140-148 after Kudo. ×2350.
Fig. 114. Planonts. ×1000.
Fig. 115. Stained Young spores. ×1500.
Fig. 116. Planonts.
Fig. 117. A planont from a host cell (fresh).
Fig. 118. A meront.
Figs. 119 to 127. Meronts of various form and stages of multiplication.
Fig. 128. The end product of schziogony.
Figs. 129 to 132. Stages in the development of the spore. ×2150.
Fig. 133. A spore with four nuclei in the girdle-like sporoplasm.
Fig. 134. A typical spore with two nuclei, the other three, undergoing degeneration.
Fig. 135. A spore treated with creosote and stained with hematoxylin.
Fig. 136. Spores similarly treated, but stained with Romanowsky and Giemsa respectively.
Fig. 137. A fresh spore.
Fig. 138. A spore showing the extruded filament. Giemsa after creosote.
Fig. 139. A spore with the extruded filament (iodine water).
Figs. 140 to 145. Different spores from a single host.
Figs. 146 and 147. Stained spores.
Fig. 148. A spore with the extruded filament viewed under a dark-field microscope. ×1200.
Fig. 149. A spore with extruded filament of *Nosema branchiale* After Nemeczek.
Fig. 150. A part of the section through the oral sucker of the metacercaria of *Gymnophallus somateriae* Lav. var. *strigatus*, showing the diffuse infiltration of the spores of *Nosema legeri*. After Dollfus.
Figs. 151 to 153. *Nosema pulicis.* After Nöller.
Fig. 151. A spore with the extruded filament. ×1300.
Fig. 152. Giemsa stained spore. ×2700.
Fig. 153. A spore stained with Heidenhan's iron hematoxylin. ×1950.
Figs. 154 to 157. Spores of *Nosema glossiphoniae.* After Schröder.
Figs. 158-177. *Nosema bombi.* After Fantham and Porter. ×1300.
Fig. 158. Planonts.
Fig. 159. Meronts.
Figs. 160 to 164. Dividing meronts.
Figs. 165 to 171. Meronts of various size, form and stages in division.
Figs. 172 to 177. Stages in spore formation.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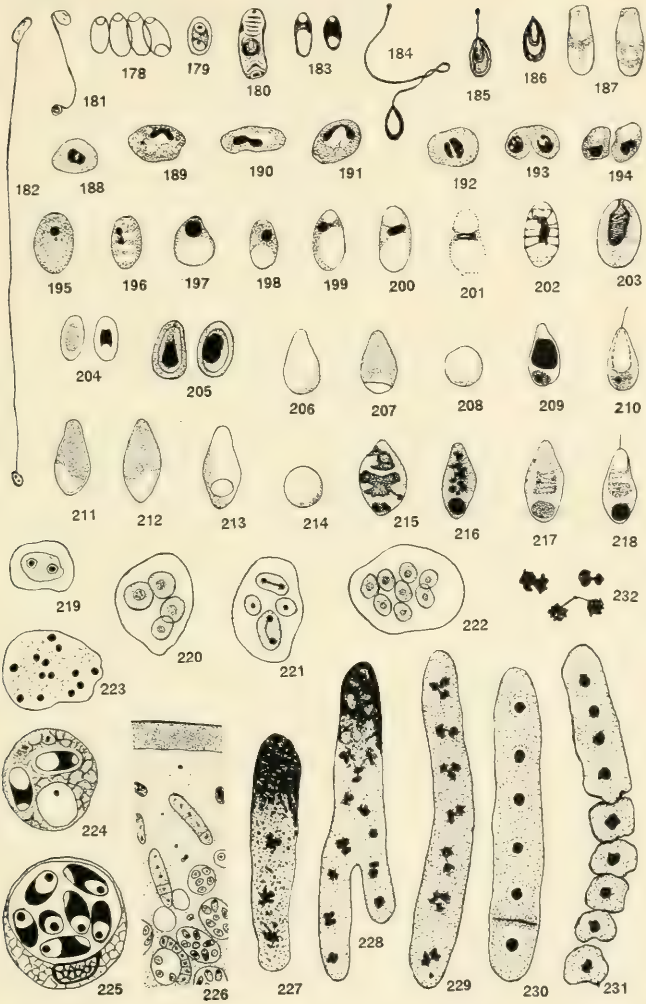
KUDO A STUDY OF THE MICROSPORIDIA PLATE IV

PLATE V

EXPLANATION OF PLATE V

Figs. 178 to 182. *Nosema ctenocephali.* After Korke. ×1800.
Fig. 178. Fresh spores. ×1200.
Figs. 179 and 180. Spores.
Figs. 181 and 182. Spores with the extruded filaments. ×600.
Figs. 183 to 186. *Nosema* sp. Ishiwata. After Ishiwata.
Fig. 183. Spores in India ink.
Fig. 184. A spore with the extruded filament.
Figs. 185 and 186. Spores showing the coiled filaments.
Fig. 187. Spores of *Nosema culicis.* After Bresslau. ×2150.
Figs. 188 to 205. *Nosema baetis.* After Kudo. ×2350.
Figs. 188 to 194. Stages in schizogony.
Figs. 195 to 202. Stages in sporogony.
Fig. 203. A spore pressed mechanically and stained with Fontana.
Figs. 204 and 205. Spores.
Figs. 206 to 210. *Nosema cyclopis.* After Kudo. ×2350.
Figs. 206 and 207. Fresh spores.
Fig. 208. End view of a fresh spore.
Fig. 209. A stained spore.
Fig. 210. A spore pressed mechanically, showing the extruded filament.
Figs. 211 to 218. *Nosema infirmum.* After Kudo. ×2350.
Figs. 211 to 213. Fresh spores.
Fig. 214. An end view of a spore.
Figs. 215 and 216. Stained young spores.
Fig. 217. A mature spore stained.
Fig. 218. A spore with its partly extruded filament.
Figs. 219 to 232. *Glugea anomala.* 219-222 after Thélohan; 223-225 after Stempell ×2250; 226-232 after Awerinzew and Fermor.
Figs. 219 to 222. Sporoblasts in development.
Fig. 223. A sporont.
Fig. 224. A secondary body with the spores.
Fig. 225. The same with numerous spores.
Fig. 226. The peripheral portion of a cyst.
Figs. 227 to 230. Stages in the development of secondary cylinders.
Fig. 231. Division of a secondary cylinder in a vacuole.
Fig. 232. Nuclei in division shown in fig. 229.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KUDO A STUDY OF THE MICROSPORIDIA PLATE V

PLATE VI

EXPLANATION OF PLATE VI

Figs. 233 to 264. *Gluega anomala.* 233-250 after Weissenberg (233-236, ×1500; 237-250 ×2500); 251-255 after Thélohan; 256-264 after Stempell. ×2250.

Fig. 233. A single schizont found in a host cell from the connective tissue of intestine of a young fish seven days after feeding infected material.

Fig. 234. An enlarged host cell with three nuclei infected by a tetranucleated schizont. Seven days after infection.

Fig. 235. An enlarged host cell undergoing degeneration. Seven days after infection.

Fig. 236. A primary cyst 41μ in diameter. Nine days after infection.

Fig. 237. Uninucleated primary cylinder.

Fig. 238. Binucleated primary cylinder.

Figs. 239 to 242. Stages in the development of secondary cylinders.

Fig. 243. Division of the secondary cylinders in a vacuole.

Figs. 244 to 247. Stages in development of multinucleated spherical bodies from the primary cylinder.

Fig. 248. Vacuole cells each with a resting nucleus.

Fig. 249. Two vacuole cells undergoing division to form sporoblasts.

Fig. 250. A later stage.

Fig. 251. A fresh spore. ×2250.

Fig. 252. A fresh spore showing the sutural line. ×2250.

Figs. 253 and 254. Stained spores.

Fig. 255. A spore with its filament extruded under the action of iodine water.

Fig. 256. A fresh spore.

Fig. 257. A spore kept in formol and alcohol for a long time and studied in water.

Figs. 258 to 261. Stages in sporulation.

Fig. 262. A schematic figure of a ripe spore. ×7000.

Figs. 263 and 264. Spores with extruded filaments under the action of iodine water.

Figs. 265 to 267. *Glugea destruens.* After Thélohan.

Fig. 265. The peripheral portion of the parasite in section.

Fig. 266. A fresh spore.

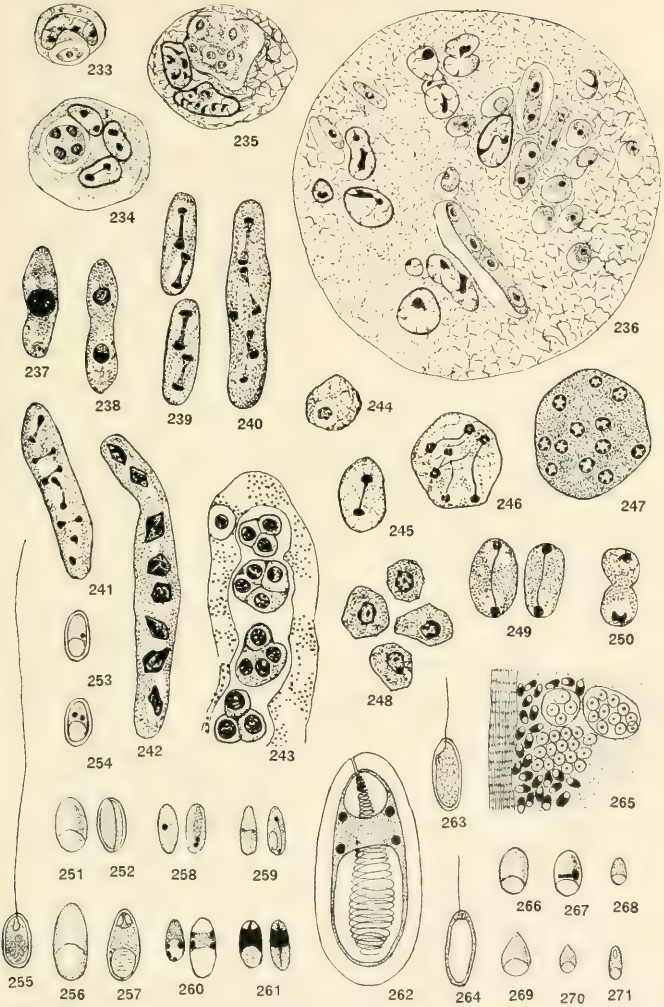
Fig. 267. A spore from a section.

Fig. 268. A spore of *Glugea ovoidea.* After Thélohan. ×1500.

Fig. 269. A spore of *Glugea acuta.* After Thélohan. ×1500.

Fig. 270. A spore of *Glugea cordis.* After Thélohan. ×1500.

Fig. 271. A spore of *Glugea depressa.* After Thélohan. ×1500.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KUDO A STUDY OF THE MICROSPORIDIA PLATE VI

PLATE VII

EXPLANATION OF PLATE VII

Figs. 272 and 273. *Glugea punctifera*. After Thélohan.

Fig. 272. Cross-section of the interfasicular connective tissue of the infected eye-muscles of *Gadus pollachius*.

Fig. 273. A fresh spore.

Figs. 274 to 295. *Glugea danilewskyi*. 274-293 after Debaisieux×2000; 294,295 after Guyénot and Naville×6266.

Fig. 274. First and second nuclear divisions in the vegetative form.

Figs. 275 and 276. The more diffused nuclear division.

Fig. 277. Second nuclear division.

Fig. 278. Further nuclear division.

Fig. 279. Forms produced by rapidly dividing nuclei.

Fig. 280. An individual with unseparated daughter nuclei.

Fig. 281. A plasmodium.

Fig. 282. A plasmodium starting to divide.

Fig. 283. Autogamic copulae in the secondary cyst.

Fig. 284. The same, showing a nuclear division of enigmatic type.

Fig. 285. Copulae and division of sporoplasts at various stages.

Fig. 286. Division into sporoblasts.

Fig. 287. Individualization of sporoblasts.

Fig. 288. Young sporoblasts.

Fig. 289. Copulae producing plasmodia.

Fig. 290. A young sporoblast. Enigmatic type.

Fig. 291. Two young spores.

Fig. 292. Two spores.

Fig. 293. Two voluminous spores.

Fig. 294. A spore with extruded filament (only part of it shown) and the spore membrane ruptured under the action of hydrochloric acid.

Fig. 295. A spore treated with hydrochloric acid and stained with Heidenhain's iron hematoxylin and Bordeaux red.

Figs. 296 to 301. *Glugea mülleri*. After Debaisieux. × about 2000.

Fig. 296. Four young vegetative stages at various phases of development.

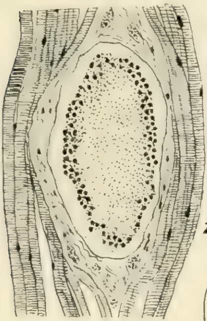
Fig. 297. Second nuclear division.

Fig. 298. A vegetative form with a large nucleus.

Fig. 299. A plasmodium.

Fig. 300. Division of a plasmodium.

Fig. 301. Autogamic copulae.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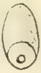
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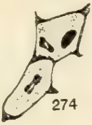
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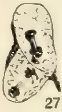
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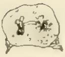
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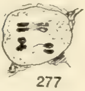
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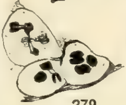
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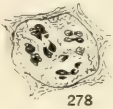
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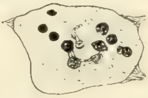
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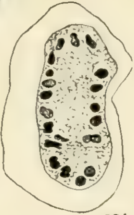
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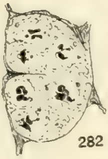
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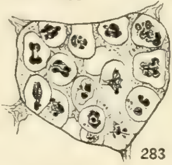
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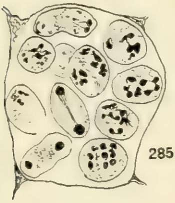
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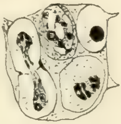
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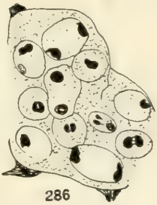
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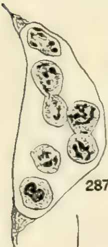
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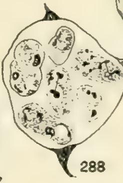
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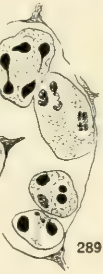
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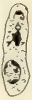
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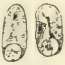
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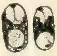
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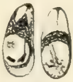
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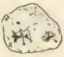
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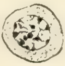
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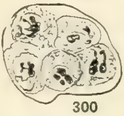
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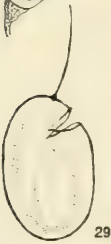
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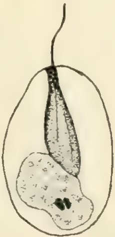
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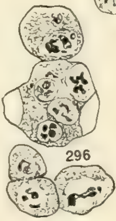
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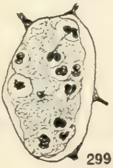
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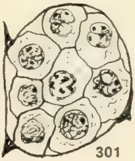
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KUDO A STUDY OF THE MICROSPORIDIA PLATE VII

PLATE VIII

EXPLANATION OF PLATE VIII

Figs. 302 to 313. *Glugea mülleri.* After Debaisieux. × about 2000.

Figs. 302 and 303. Division of zygotes into sporoblasts.

Fig. 304. Young spores.

Figs. 305 to 313. Spores.

Figs. 314 to 316. *Glugea stephani.* 314, 316 after Johnstone; 315 after Woodcock.

Fig. 314. Part of a transverse section of the infected gut wall of *Pleuronectes platessa*. ×15.

Fig. 315. Part of a cross-section of an infected gut wall showing the peripheral part of the tumor. ×500.

Fig. 316. Spores. ×2500

Fig. 317. A spore of *Glugea shiplei*. After Drew ×2000

Figs. 318 to 335. *Glugea hertwigi*. 318-330 after Weissenberg ×2500; 331-335 after Schrader.

Fig. 318. A sporoblast with its nucleus at one end.

Fig. 319. A sporoblast with its centrally located nucleus.

Fig. 320. A sporoblast after the formation of the membrane.

Fig. 321. A more advanced sporoblast.

Fig. 322. A fresh spore.

Fig. 323. A spore in water to which a few drops of Flemming's solution were added.

Fig. 324. Spores.

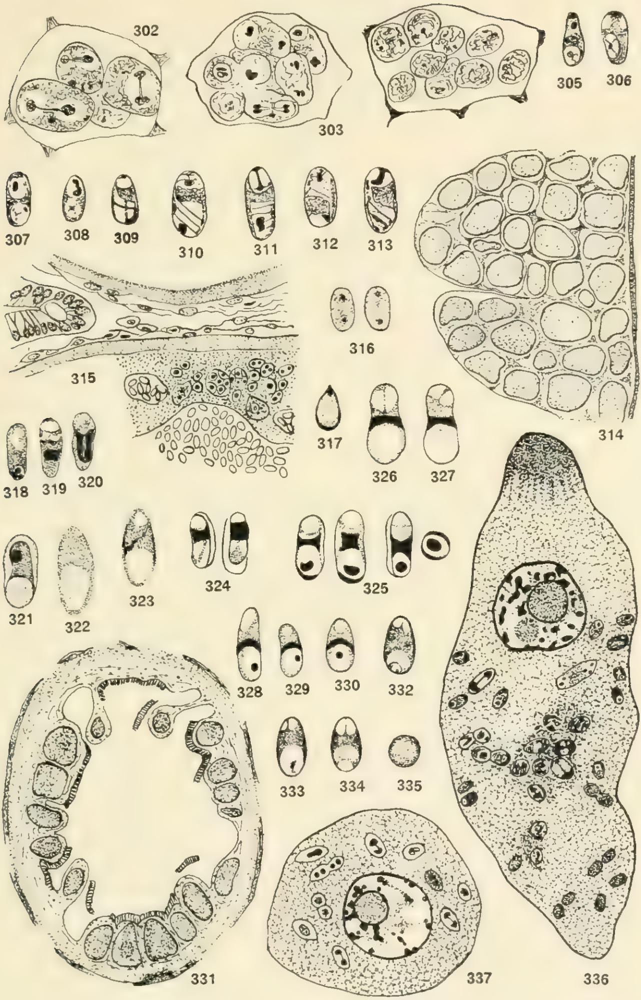
Fig. 325. Spores showing metachromatic granules.

Figs. 326 to 330. Spores.

Fig. 331. Cross-section of the infected intestine of a smelt. ×20.

Figs. 322 to 335. Spores.

Figs. 336 and 337. Sections of two extracellular forms of *Lankesteria ascidiae* infected by *Perezia lankesteriae*. After Léger and Duboscq. ×1345.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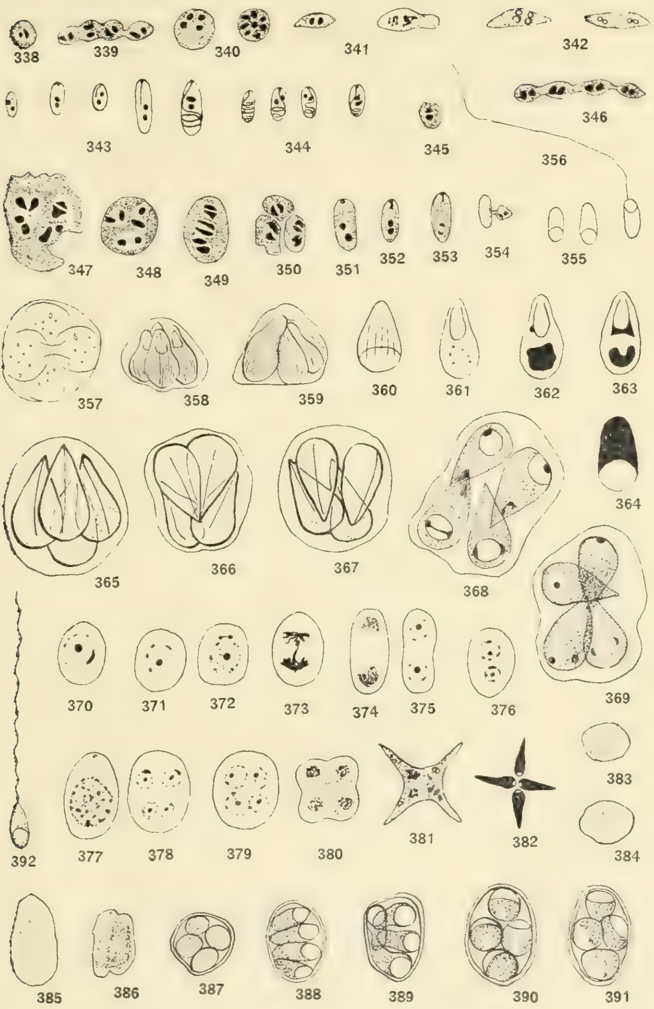
KUDO A STUDY OF THE MICROSPORIDIA PLATE VIII

PLATE IX

EXPLANATION OF PLATE IX

Figs. 338 to 344. *Perezia mesnili*. After Paillot. ×1200.
Figs. 338 to 340. Stages in schizogony.
Figs. 341 and 342. Stages in sporogony.
Fig. 343. Stages in the development of spore.
Fig. 344. Spores.
Figs. 345 to 356. *Perezia legeri*. After Paillot. ×1200.
Figs. 345 to 350. Stages in schizogony.
Figs. 351 to 353. Stained spores.
Fig. 354. A spore in the smear of fat body, in which the sporoplasm is seen leaving the spore.
Fig. 355. Fresh spores.
Fig. 356. A spore with the extruded filament.
Figs. 357 to 364. *Gurleya tetraspora*. After Doflein.
Fig. 357. A sporont with four sporoblasts.
Figs. 358 and 359. Panpsoroblasts containing fully formed spores.
Fig. 360. A spore in fresh condition.
Fig. 361. A spore treated with acetic acid.
Figs. 362 to 364. Spores stained with safranin.
Figs. 365 to 369. *Gurleya legeri*. After Mackinnon.
Figs. 365 to 367. Sporonts in fresh condition, showing the spores.
Figs. 368 and 369. Typical sporoblasts stained with Delafield hematoxylin.
Figs. 370 to 382. *Gurleya francottei*. After Léger and Duboscq.
Figs. 370 to 376. Stages in schizogony.
Figs. 377 to 381. Stages in sporogony.
Fig. 382. Fully formed spores.
Figs. 383 to 392. *Gurleya richardi*. After Cépède.
Figs. 383 to 386. Schizonts.
Figs. 387 to 389. Pansporoblasts with microspores.
Figs. 390 and 391. Pansporoblasts with macrospores.
Fig. 392. A spore with the filament extruded under the action of physiological salt solution ×1800.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KUDO A STUDY OF THE MICROSPORIDIA PLATE IX

PLATE X

EXPLANATION OF PLATE X

Figs. 393 to 434. *Thelohania giardi*. 393-402 after Henneguy and Thélohan; 403, 404 after Thélohan; 405-434 after Mercier. (×1800).

Fig. 393. A young pansporoblast in fresh state.

Figs. 394 to 397. Stages showing division of the sporonts.

Figs. 398 and 399. Changes in the sporoblasts.

Fig. 400. A pansporoblast with eight normal spores.

Fig. 401. A normal spore.

Fig. 402. Two abnormal spores.

Fig. 403. A spore in fresh condition.

Fig. 404. A spore with its filament extruded under the action of sulphuric acid.

Figs. 405 to 409. Schizonts.

Figs. 410 to 413. Four stages in fusion of two isogametes.

Fig. 414. The copula in which the syncharion is expelling the chromidia.

Figs. 415 to 419. Different stages in the division of the syncharion.

Figs. 420 to 423. Further changes in sporonts.

Figs. 424 and 425. Pansporoblasts with eight sporoblasts.

Figs. 426 to 432. Stages in development of the spores. ×2400.

Fig. 433. A spore. ×2400.

Fig. 434. A spore whose sporoplasm nuclei undergoing division. ×2400.

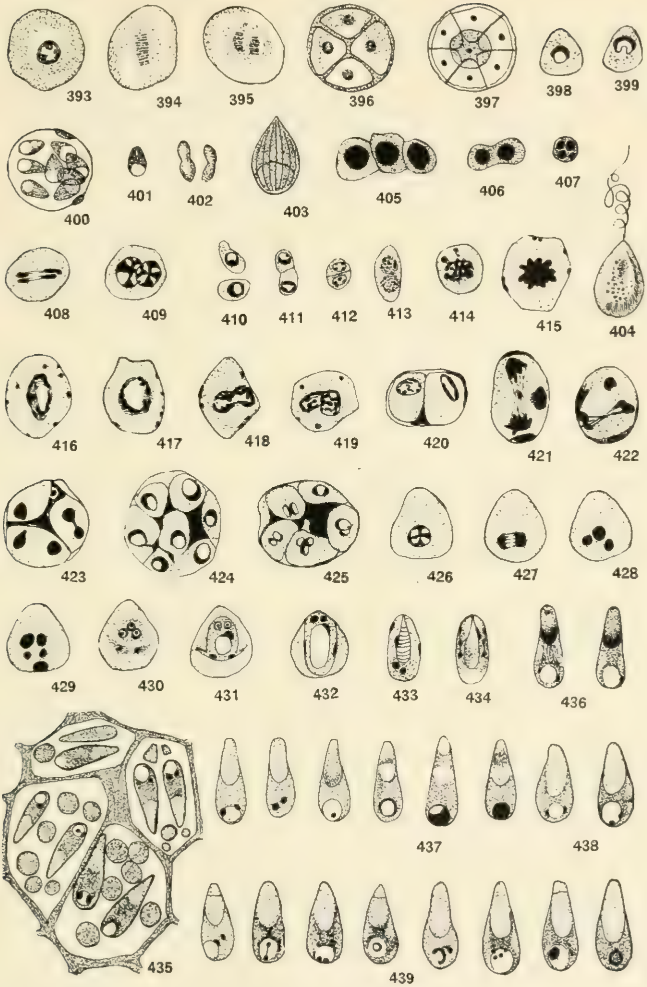
Figs. 435 to 439. *Thelohania acuta*. After Schröder.

Fig. 435. Section through pansporoblasts.

Fig. 436. Spores stained with Mallory's method.

Fig. 437. Six spores stained with Delafield's hematoxylin.

Figs. 438 and 439. Ten spores stained with methylene blue rec. Ehrlich.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KUDO A STUDY OF THE MICROSPORIDIA PLATE X

PLATE XI

EXPLANATION OF PLATE XI

Fig. 440. Spores of *Thelohania virgula*. After Pfeiffer. ×1500.

Figs. 441 to 443. Spores of *Thelohania octospora*. 441, 442 after Thélohan; 443 after Goodrich ×2000.

Fig. 441. A fresh spore.

Fig. 442. A spore with its filament extruded under the action of ether.

Fig. 443. A spore treated with iodine.

Figs. 444 and 445. *Thelohania contejeani*. After Henneguy and Thélohan.

Fig. 444. A pansporoblast.

Fig. 445. A spore.

Figs. 446 to 448. *Thelohania macroçystis*. After Garbini from Gurley.

Figs. 446 and 447. Panpsoroblasts (?).

Fig. 448. Spores.

Figs. 449 to 460. *Thelohania mulleri*. 449-458 after Stempell ×2250; 459, 460 after Léger and Hesse.

Fig. 449. A young meront and six meronts undergoing multiplication in fresh condition.

Fig. 450. A meront.

Figs. 451 and 452. Stages corresponding to Fig. 449 in stained state.

Figs. 453 and 454. Stages in sporogony in fresh state.

Figs. 455 and 456. Similar stages from stained preparations.

Fig. 457. A normal fresh spore.

Fig. 458. A spore with its filament extruded under the action of iodine alcohol.

Fig. 459. A fully grown sporont. ×2000.

Fig. 460. A fresh spore. ×2200.

Figs. 461 to 472. *Thelohania varians*. After Debaisieux. ×about 2000.

Fig. 461. Uninucleate meront.

Fig. 462. Meronts undergoing division.

Fig. 463. Two meronts after division.

Fig. 464. Further nuclear division in meronts.

Fig. 465. Meronts in a binucleate host cell.

Fig. 466. Elongated vegetative forms.

Fig. 467. Another elongated form.

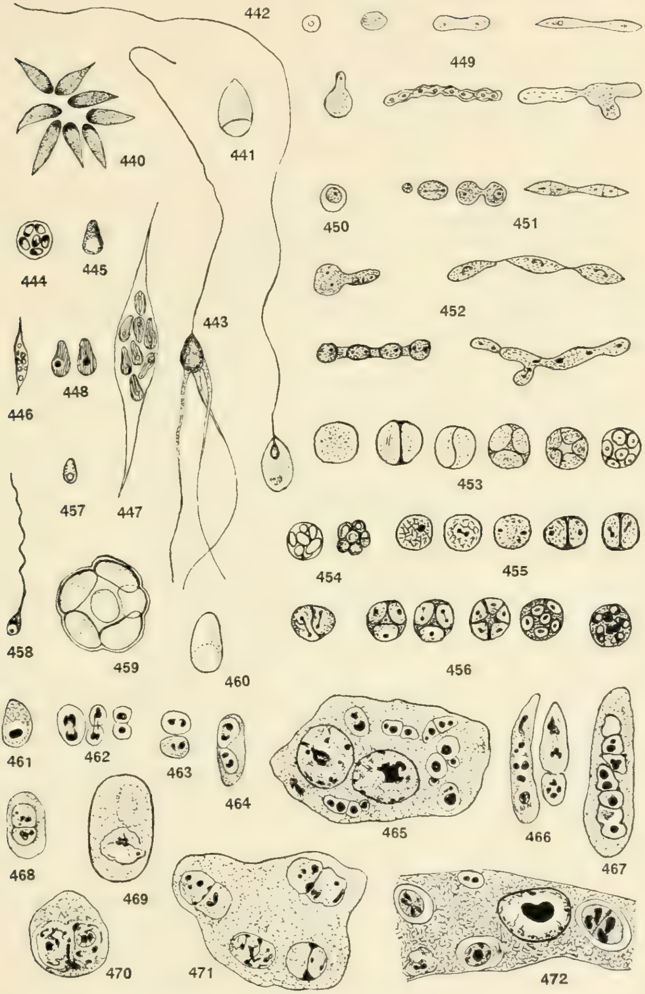
Fig. 468. Final stage in vegetative multiplication.

Fig. 469. Second aspect of schizogony. A voluminous spore with a large nucleus.

Fig. 470. A plasmodium with two nuclei.

Fig. 471. A multinucleate plasmodium.

Fig. 472. Third aspect of schizogony showing isolated individuals.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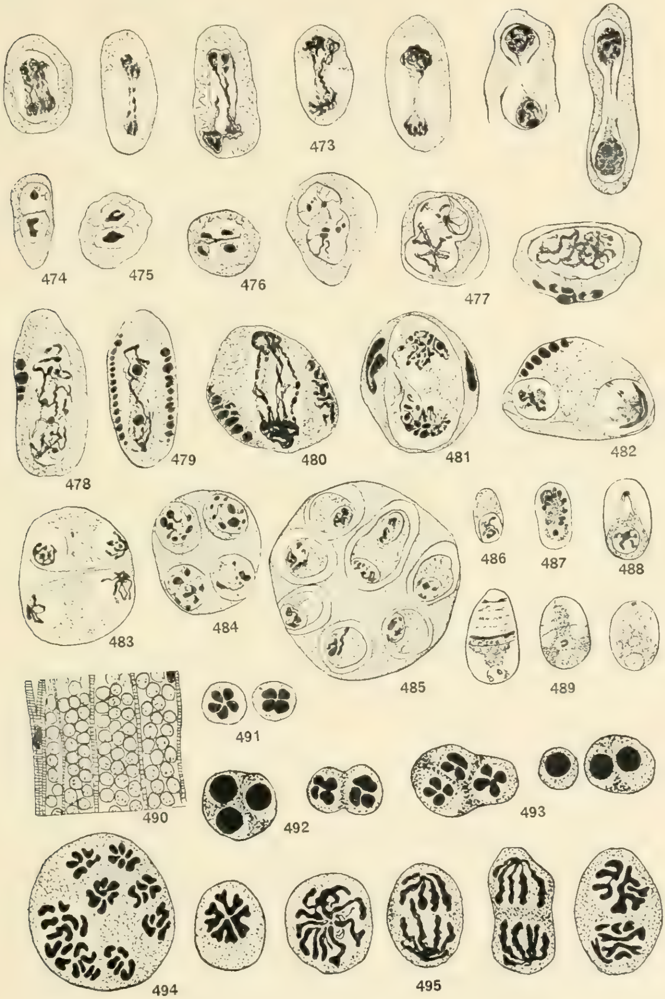
KUDO A STUDY OF THE MICROSPORIDIA PLATE XI

PLATE XII

EXPLANATION OF PLATE XII

Figs. 473 to 489. *Thelchania varians*. After Debaisieux. ×about 2000.
Fig. 473. Seven stages in "amitotic" division.
Fig. 474. The expulsion of chromatic granules of the nuclei.
Figs. 475, 476 and 477. Stages in the formation of zygotes.
Fig. 478. A zygote or sporont with a large nucleus.
Figs. 479 to 482. The first nuclear divisions in sporont.
Figs. 483 and 484. Formation of four nucleated stage.
Fig. 485. Pansporoblast with eight sporoblasts.
Fig. 486. A young spore contained in a pansporoblast.
Fig. 487. A young spore containing a metachromatic mass.
Fig. 488. A spore showing numerous granules and metachromatic mass.
Fig. 489. Three spores with spirally coiled filament.
Figs. 490 to 495. *Thelohania maenadis*. After Pérez. ×2000.
Fig. 490. The infected muscle fibers of *Carcinus maenas*.
Figs. 491 to 494. Meronts of various size.
Fig. 495. Five stages in schizogony.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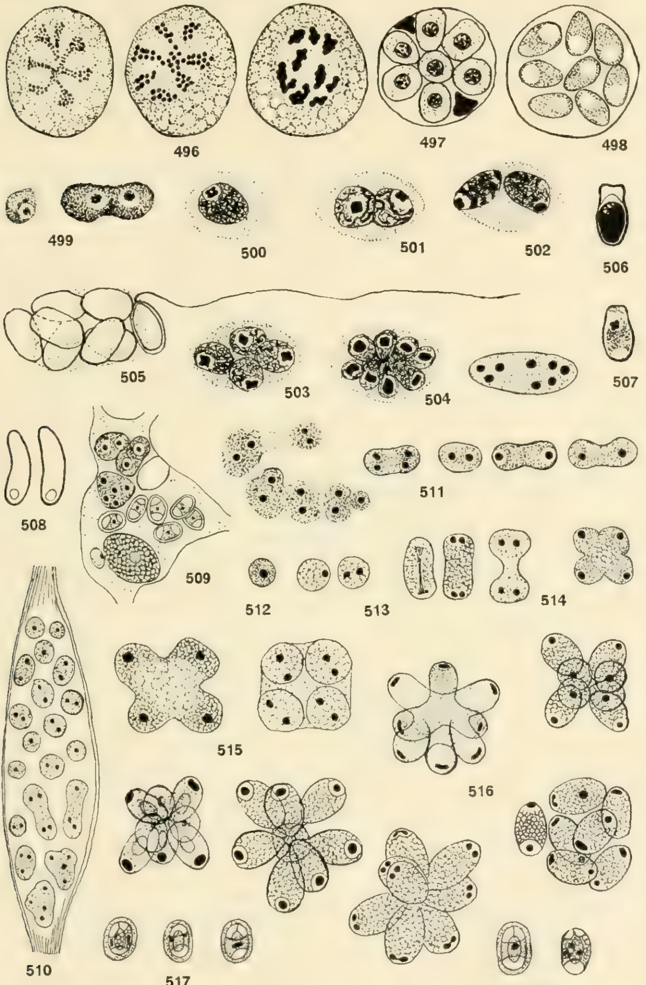
KUDO A STUDY OF THE MICROSPORIDIA PLATE XII

PLATE XIII

EXPLANATION OF PLATE XIII

Figs. 496 to 498. *Thelohania maenadis*. After Pérez. ×2000.
Fig. 496. Three stages in sporogony.
Fig. 497. Pansporoblast with sporoblasts.
Fig. 498. A fully developed pansporoblast in fresh state.
Figs. 499 to 507. *Thelohania legeri*. After Hesse. ×1800.
Fig. 499. Meronts.
Figs. 500 to 504. Stages in sporogony.
Fig. 505. A pansporoblast with one of the spores with its extruded filament, in fresh state.
Fig. 506. A spore stained with iron hematoxylin.
Fig. 507. A spore stained after Romanowsky.
Fig. 508. Spores of *Thelohania braziliensis*. After Lutz and Splendore.
Figs. 509 to 518. *Thelohania chaetogastris*. After Schröder. ×2000.
Fig. 509. Infected connective tissue of the host with isolated spores and three schizonts. ×1000.
Fig. 510. The infected muscle fibers with numerous schizonts. ×1000.
Figs. 511 and 512. Stages in schizogony.
Figs. 513 to 516. Stages in sporogony.
Figs. 517 and 518. Five stained spores.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KUDO A STUDY OF THE MICROSPORIDIA PLATE XIII

PLATE XIV

EXPLANATION OF PLATE XIV

Fig. 519. A pansporoblast and a spore of *Thelohania giraudi* in fresh state. After Léger and Hesse. ×2000.

Figs. 520 to 524. *Thelohania ovicola.* After Auerbach.

Fig. 520. A sporont and a pansporoblast.

Fig. 521. A pansporoblast in fresh condition.

Fig. 522. A spore in glygerine.

Fig. 523. A spore with its extruded filament.

Fig. 524. Stained spores.

Figs. 525 to 531. *Thelohania bracteata.* 525-528 after Strickland ×1400; 529-531 after Debaisieux and Gastaldi ×2250.

Fig. 525. Peripheral portion of the parasitic mass ×470.

Fig. 526. A newly formed sporont and the changes observed therein.

Fig. 527. A fully formed pansporoblast in fresh state.

Fig. 528. A spore with its extruded filament.

Fig. 529. A fully formed pansporoblast.

Fig. 530. A spore.

Fig. 531. Macrospores.

Figs. 532 to 542. *Thelohania fibrata.* 532-539 after Strickland ×1400; 540-542 after Debaisieux and Gastaldi ×2250.

Fig. 532. Dividing meronts.

Fig. 533. A newly formed sporont with scattered chromatic substance.

Figs. 534 to 536. Stages in sporogony.

Fig. 537. A spore stained with hematoxylin, showing the polar capsule. ×1600.

Fig. 538. A macrospore. ×1600.

Fig. 539. Spores with extruded polar filaments. ×1600.

Fig. 540. A pansporoblast with sporoblasts.

Fig. 541. A fresh spore.

Fig. 542. A macrospore.

Figs. 543 to 548. *Thelohania multispora.* 543-547 after Strickland ×1400; 548 after Debaisieux and Gastaldi ×2250.

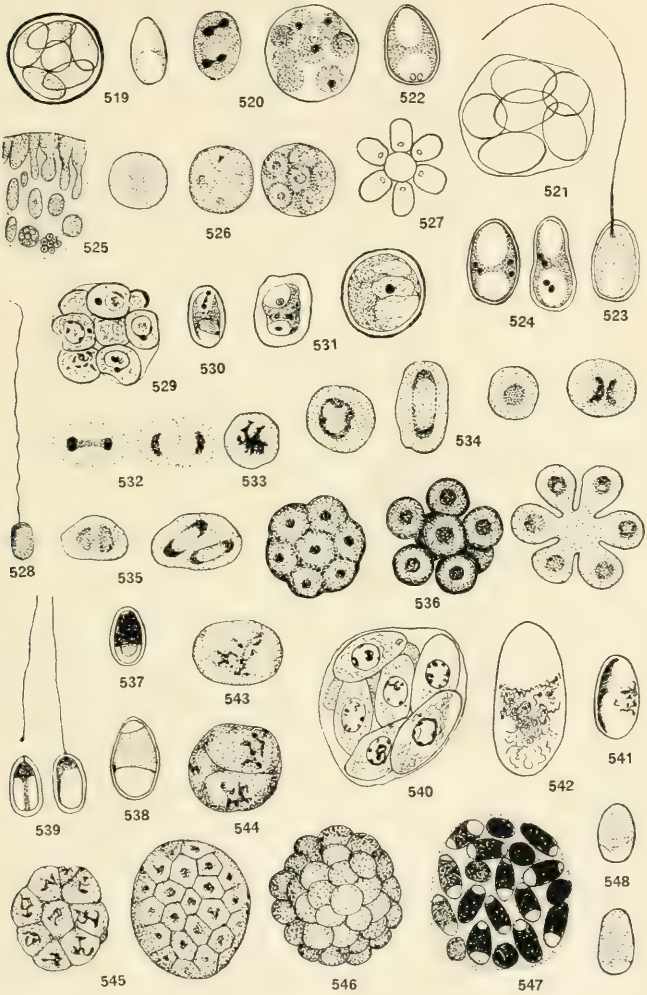
Fig. 543. A sporont.

Figs. 544 and 545. Formation of sporoblasts in sporonts.

Fig. 546. Unstained pansporoblast.

Fig. 547. Sectioned pansporoblast.

Fig. 548. Fresh spores.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KUDO A STUDY OF THE MICROSPORIDIA PLATE XIV

PLATE XV

EXPLANATION OF PLATE XV

Figs. 549 to 552. *Thelohania ovata*. After Dunkerly.

Fig. 549. A trophozoite budding off uninucleated bodies. ×2000.

Fig. 550. Sporoblasts. ×1000.

Fig. 551. Two microspores. ×1000.

Fig. 552. A pansporoblast with macrospores. ×1250.

Figs. 553 to 557. *Thelohania corethrae*. After Schuberg and Rodriguez.

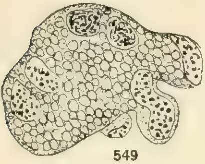
Figs. 553 and 554. Stages in schizogony.

Figs. 555 and 556. Stages in sporogony.

Fig. 557. Three spores stained.

Fig. 558. Eight stages in sporogony of *Thelohania* sp. After Nöller. ×2500.

Fig. 559. Stages in schizogony of *Thelohania opacita*. After Kudo. ×2300.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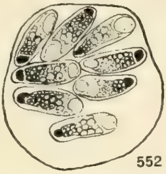
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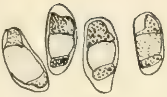
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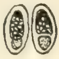
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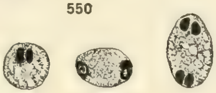
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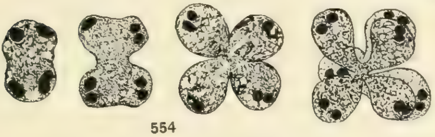
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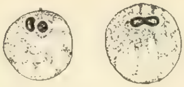
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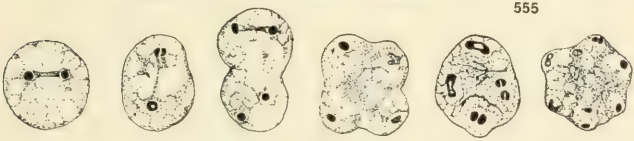
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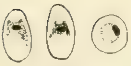
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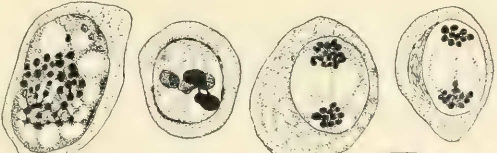

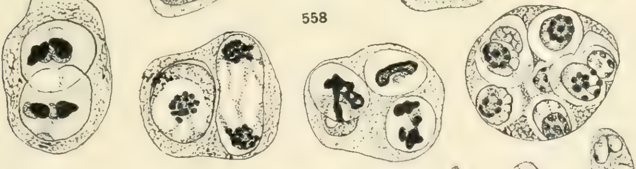
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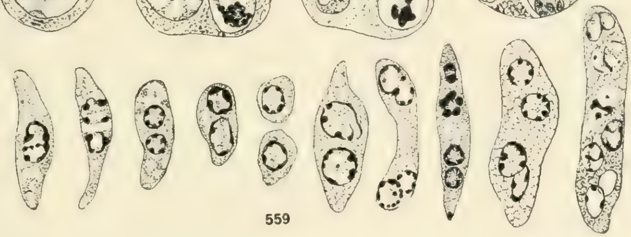
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KUDO A STUDY OF THE MICROSPORIDIA PLATE XV

PLATE XVI

EXPLANATION OF PLATE XVI

Figs. 560 to 570. *Thelohania opacita.* After Kudo.

Fig. 560. Five stages in the formation of the sporont. ×2300.

Fig. 561. A sporont whose nucleus undergoing the first division. ×2300.

Figs. 562 and 563. Stages in sporogony. ×2300.

Fig. 564. A pansporoblast with eight nuclei. ×2300.

Fig. 565. Three fully formed pansporoblasts. ×2300.

Fig. 566. A tetrasporous pansporoblast in fresh state. ×2360.

Fig. 567. Three normal octosporous pansporoblasts in fresh state. ×2360.

Fig. 568. Various spores in fresh state. ×2360.

Fig. 569. Stained spores. ×2300.

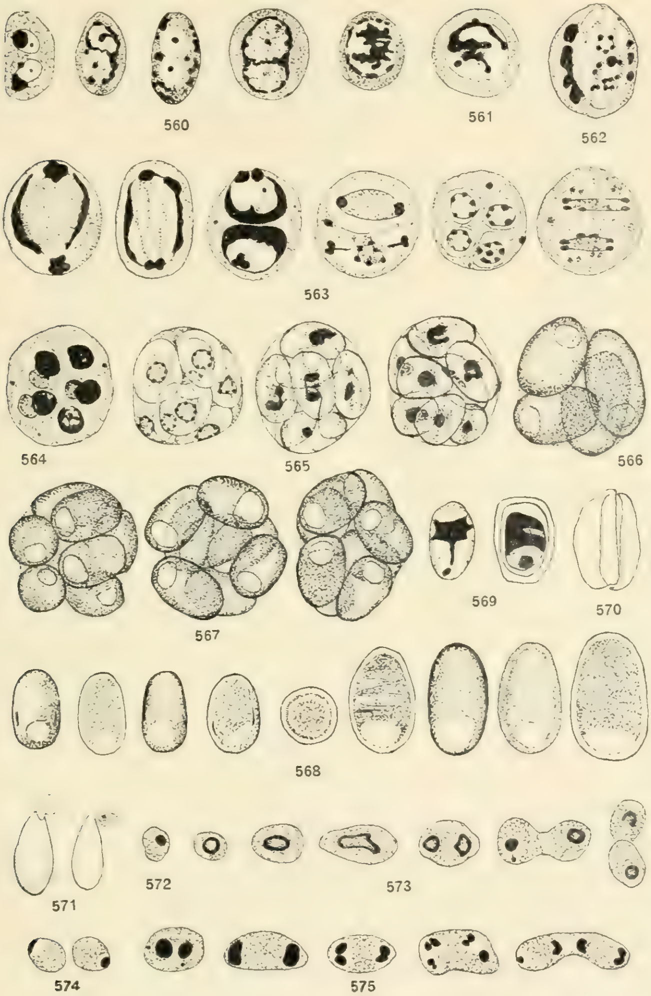
Fig. 570. A spore compressed, showing two valves in the membrane. ×3200.

Figs. 571 to 575. *Stempellia magna.* After Kudo. ×2300.

Fig. 571. Spores from which the sporoplasms are escaping in the midgut in experimentally infected larvae (24 hours and 40 hours respectively). ×1000.

Fig. 572. A young schizont found in peristomachal fat body, 24 hours after feeding.

Figs. 573 to 575. Stages in schizogony.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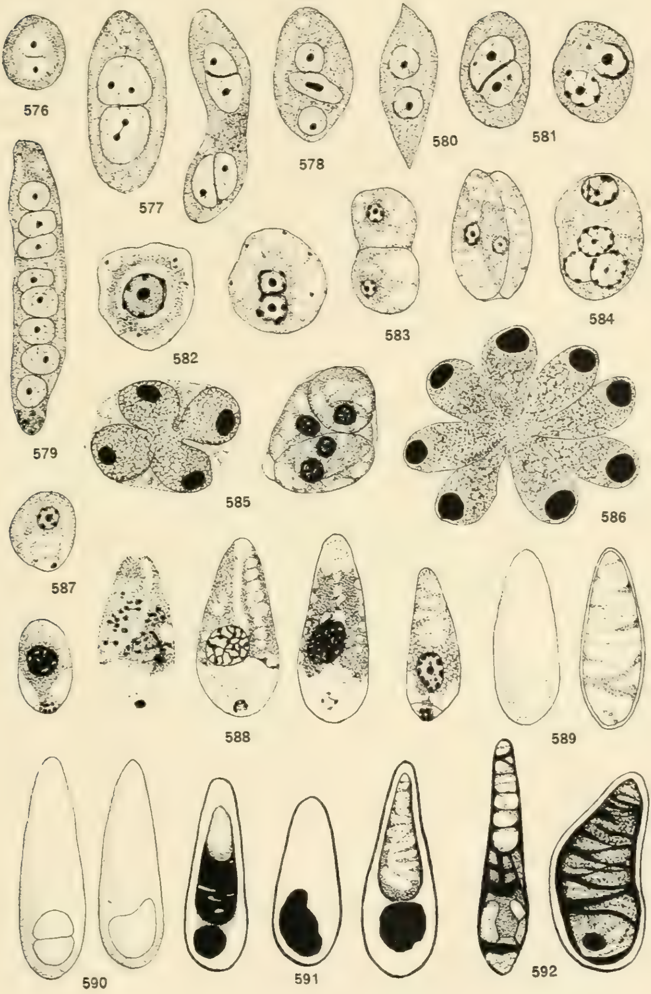
KUDO A STUDY OF THE MICROSPORIDIA PLATE XVI

PLATE XVII

EXPLANATION OF PLATE XVII

Figs. 576 to 592. *Stempellia magna*. After Kudo ×2300 unless otherwise stated.
Figs. 576 to 579. Stages in schizogony found in naturally infected host larvae.
Fig. 580. The last stage in schizogony.
Fig. 581. Stages in the fusion of the two nuclei.
Fig. 582. A sporont.
Fig. 583. Three stages in the development of disporous pansporoblast.
Figs. 584 and 585. Stages in the formation of tetrasporous pansporoblast. 585×2360.
Fig. 586. An octosporous sporont. ×2360.
Fig. 587. A sparoblast.
Fig. 588. Five stages in the development of spore. ×2360.
Figs. 589 and 590. Fresh spores.
Fig. 591. Three spores stained with methylene blue.
Fig. 592. Abnormal spores.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KUDO A STUDY OF THE MICROSPORIDIA PLATE XVII

PLATE XVIII

EXPLANATION OF PLATE XVIII

Figs. 593 to 596. *Stempellia magna.* After Kudo. ×2360.

Fig. 593. A spore with the extruded filament.

Figs. 594 and 595. Spores with extruded filaments under low magnification (×900) and higher magnification.

Fig. 596. Spores mechanically compressed and kept in a mixture of Lugol and gum arabic

Figs. 597 and 598. *Plistophora typicalis.* After Thélohan.

Fig. 597. Section of infected muscle fibers of *Cottus scorpius.*

Fig. 598. A fresh spore and a spore with its filament extruded under the action of iodine water. ×2250.

Figs. 599 and 600. *Plistophora acerinae.* After Vaney and Conte.

Fig. 599. A pansporoblast.

Fig. 600. A spore with its filament extruded under the action of iodine water.

Figs. 601 to 607. *Plistophora stegomyiae.* After Marchoux, Salimbeni and Simond.

Fig. 601. Four colorless reniform spores.

Fig. 602. Two brown reniform spores.

Fig. 603. Three colorless pyriform spores.

Fig. 604. Two brown pyriform spores.

Figs. 605 and 606. Stages in the development of colorless spores.

Fig. 607. A plasmodium with young spores.

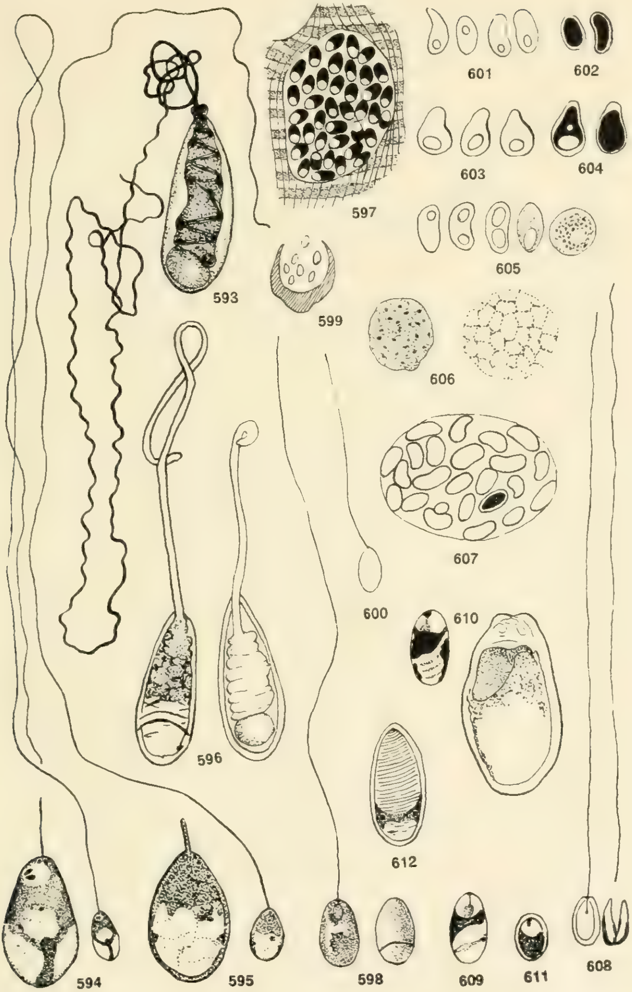
Fig. 608. Spores of *Plistophora simulii* β form. After Lutz and Splendore.

Fig. 609. A spore of *Plistophora simulii* γ form. After Debaisieux and Gastaldi.

ig. 610. A microspore and a macrospore of *Plistophora simulii* δ form. After Debaisieux and Gastaldi.

Fig. 611. A spore of *Plistophora labrorum.* After Le Danois. ×1200.

Fig. 612. A spore of *Plistophora elegans.* After Auerbach.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KUDO A STUDY OF THE MICROSPORIDIA PLATE XVIII

PLATE XIX

EXPLANATION OF PLATE XIX

Figs. 613 to 616. Spores of *Plistophora macrospora*. After Léger and Hesse. ×2500.

Fig. 613. A fresh spore.

Fig. 614. A spore treated with silver impregnation method.

Fig. 615. A spore fixed with osmic acid and stained with iron hematoxylin, showing the nucleus of the sporoplasm.

Fig. 616. A spore fixed and stained with picro-carmin. Half-schematic.

Figs. 617 and 618. *Plistophora intestinalis*. After Chatton.

Fig. 617. Infected epithelial cells of the host.

Fig. 618. A fresh spore.

Figs. 619 to 622. *Plistophora hippoglossoideos*. After Bosanquet.

Fig. 619. Fragmentation of individuals.

Fig. 620. Division stages in the formation of spores.

Fig. 621. Spores and sporoblasts.

Fig. 622. Four spores. ×2000.

Figs. 623 to 630. *Plistophora longifilis*. After Schuberg.

Fig. 623. A multinucleated pansporoblast.

Fig. 624. A pansporoblast with numerous sporoblasts.

Fig. 625. A pansporoblast with young macrospores.

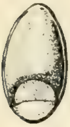
Fig. 626. A pansporoblast with mature microspores.

Fig. 627. A sporoblast.

Fig. 628. Young spores.

Fig. 629. A mature spore.

Fig. 630. Mature spores.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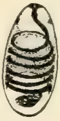
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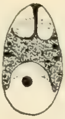
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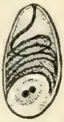
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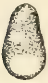
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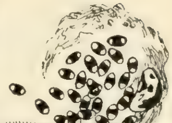
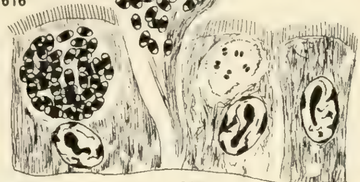
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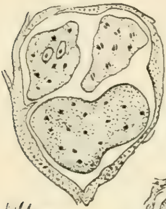
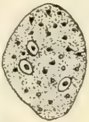
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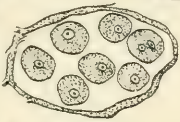
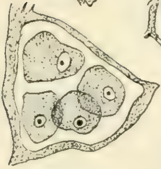
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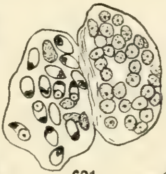
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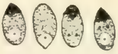
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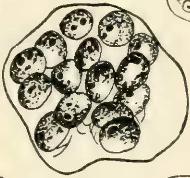
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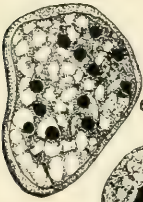
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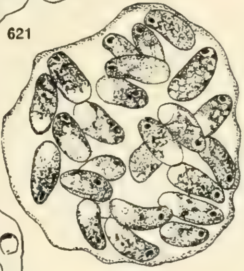
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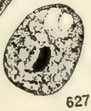
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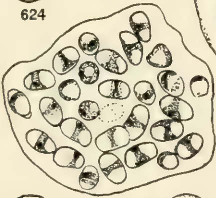
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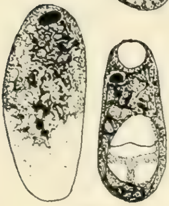
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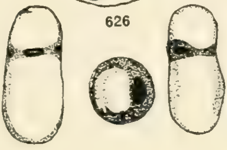
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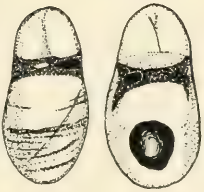
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KUDO A STUDY OF THE MICROSPORIDIA PLATE XIX

PLATE XX

EXPLANATION OF PLATE XX

Figs. 631 and 632. Spores of *Plistophora longifilis*. After Schuberg.
Fig. 631. A mature macrospore.
Fig. 632. A spore with its extruded filament.
Figs. 633 to 638. *Plistophora* sp. After Mercier.
Fig. 633. A meront.
Fig. 634. Sporonts.
Fig. 635. Pansporoblast with spores.
Fig. 636. A fresh spore showing the sutural line of shell-valves. ×1200.
Fig. 637. A spore treated with nitric acid, exhibiting the polar capsule. ×1200.
Fig. 638. A spore with its filament extruded under similar treatment. ×1200.
Figs. 639 and 640. Spores of the genus Cocconema. After Léger and Hesse.
Fig. 639. Stained spores. ×1000.
Fig. 640. A fresh spore and a spore with extruded filament. ×3000.
Fig. 641. A portion of a cyst of *Cocconema stempelli*. After Pérez. ×1000.
Figs. 642 to 644. Spores of *Mrazekia argoisi*. After Léger and Hesse. ×1750.
Fig. 642. A spore.
Fig. 643. A spore with extruded "manubrium."
Fig. 644. A spore with extruded filament.
Fig. 645. A spore of *Mrazekia brevicauda*. After Léger and Hesse. ×1750.
Fig. 646. A spore of *Mrazekia stricta*. After Léger and Hesse. ×1750.
Figs. 647 to 651. *Mrazekia caudata*. 647-650 after Mrazek; 651 after Léger and Hesse.
Figs. 647 and 648. Four stages of "Myxocystis" from Limnodrilus.
Fig. 649. Infected lymphocyte from Potamothrix.
Fig. 650. Stained spores.
Fig. 651. A spore. ×1750.
Fig. 652. A host cell infected by *Mrazekia mrazeki*. After Hesse. ×700.

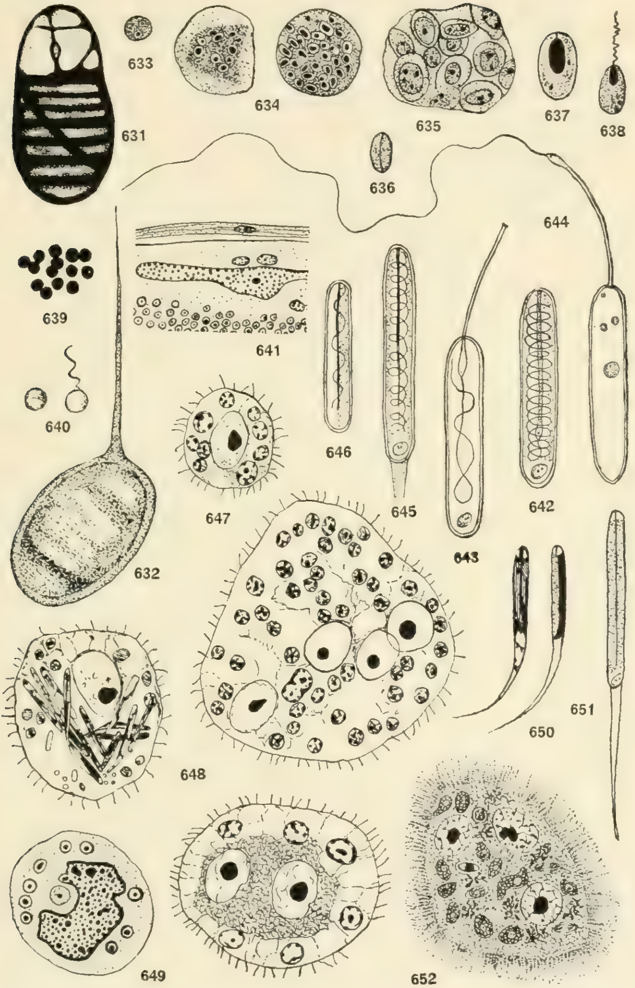


PLATE XXI

EXPLANATION OF PLATE XXI

Figs. 653 to 655. *Mrazekia mrazeki.* After Hesse.

Fig. 653. Abnormal spores. ×1500.

Fig. 654. A normal spore.

Fig. 655. A spore with its polar filament extruded.

Fig. 656. A spore of *Mrazekia tetraspora.* After Léger and Hesse. ×3000.

Fig. 657. Spores of *Mrazekia bacilliformis.* After Léger and Hesse. ×1000.

Figs. 658 to 663. *Octosporea muscae domesticae.* 658, 559 after Flu; 660-663 after Chatton and Krempf.

Fig. 658. Epithelial cells of the gut of a host infected by the microsporidian.

Fig. 659. A pansporoblast and a spore.

Fig. 660. Stages in schizogony.

Fig. 661. Stages in sporogony.

Fig. 662. Stages in the development of spores.

Fig. 663. A spore with its extruded filament.

Fig. 664. A spore of *Octosporea monospora.* After Chatton and Krempf.

Fig. 665. Spores of *Spironema octospora.* One spore is magnified 3000 times, the rest 1000 times. After Léger and Hesse.

Fig. 666. Spores of *Toxonema vibrio.* One spore is mgnified 3000 times, the rest 1000 times. After Léger and Hesse.

Fig. 667. Fresh and stained spores of *Telomyxa glugeiformis.* After Léger and Hesse. ×3000.

Fig. 668. Spores of Gen. et spec. incert. (Fritsch) from the abdomen of *Ceriodaphnia quadrangula.* After Fritsch. ×750.

Figs. 669 and 670. Pansporoblasts (?) of Gen. et spec. incert. (Christophers). 669 after Christophers; 670 after Nicholson. ×1000.

Fig. 671. Spores of Gen. et spec. incert. (Linton). After Linton. ×700.

Fig. 672. Part of the host gut epithelium showing the spores of Gen. et spec. incert. (Grassi). After Grassi.

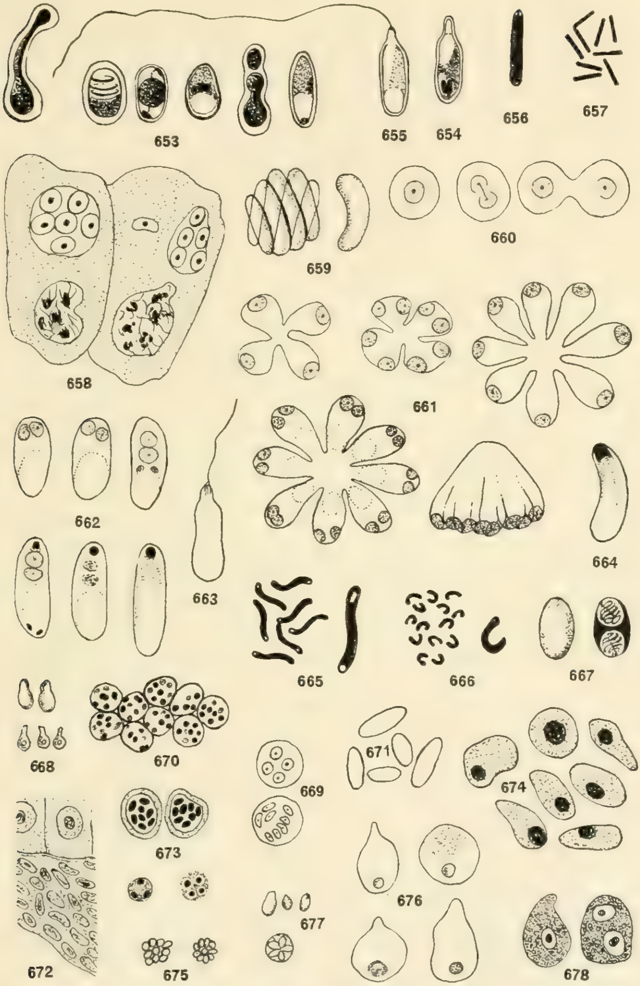
Fig. 673. Pansporoblasts of Gen. et spec. incert. (Grassi). After Grassi.

Fig. 674. Spores of Gen. incert. *schmeili.* After Pfeiffer. ×850.

Figs. 675 and 676. Spores of Gen. incert. *holopedii.* After Fritsch 675×400; 676×900.

Fig. 677. Three spores and a pansporoblast. ×750.

Fig. 678. Genus incert. *geophili.* After Crawley.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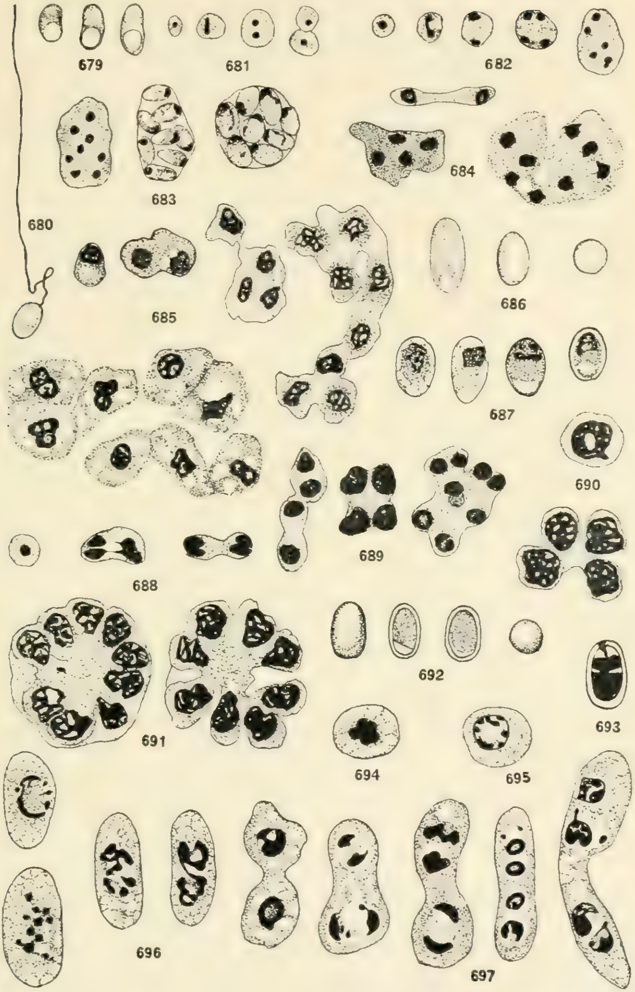
KUDO A STUDY OF THE MICROSPORIDIA PLATE XXI

PLATE XXII

EXPLANATION OF PLATE XXII

Figs. 679 to 683. *Thelohania reniformis*. After Kudo and Hetherington. ×2200.
Fig. 679. Fresh spores.
Fig. 680. A spore with its polar filament extruded.
Fig. 681. Stages in schizogony.
Figs. 682 and 683. Stages in sporogony.
Figs. 684 to 687. *Thelohania mutabilis*. After Kudo. ×2400.
Fig. 684. Stages in schizogony.
Fig. 685. Stages in sporogony.
Fig. 686. Fresh spores.
Fig. 687. Stained spores.
Figs. 688 to 693. *Thelohania baetica*. After Kudo. ×2400.
Figs. 688 and 689. Stages in schizogony.
Figs. 690 and 691. Stages in sporogony.
Fig. 692. Fresh spores.
Fig. 693. Stained spore. ×3200.
Figs. 694 to 697. *Thelohania legeri*. After Kudo. ×3200.
Figs. 694 and 695. Young schizonts.
Fig. 696. Five stages in the early phase of the schizogony.
Fig. 697. Four stages in the formation of sporont mother cells.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KUDO A STUDY OF THE MICROSPORIDIA PLATE XXII

PLATE XXIII

EXPLANATION OF PLATE XXIII

Figs. 698 to 727. *Thelohania leberi.* After Kudo. 698-719, from section preparations; 720-727, from smears. 698-719, ×3200; 720-727, ×2360.

Fig. 698. A tetranucleated schizont.

Fig. 699. A binucleated sporont mother cell.

Figs. 700 to 702. Stages in the division of the sporont mother cell.

Fig. 703. A binucleated schizont at the end of the schizogony.

Figs. 704 and 705. Stages in the fusion of the two nuclei.

Fig. 706. A sporont.

Figs. 707 to 713. Stages in the first nuclear division of the sporont.

Figs. 714 to 716. Stages in the second nuclear division of the sporont.

Figs. 717 to 719. Stages in the formation of octosporoblastic pansporoblast.

Figs. 720 to 722. Stages in the third nuclear division of the sporont as seen in a very thin smear.

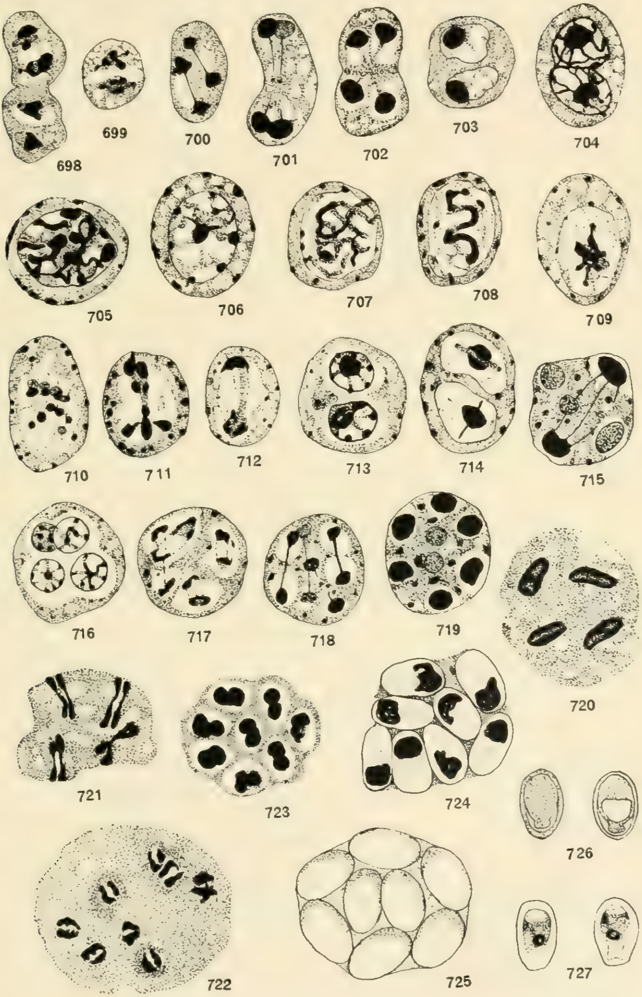
Fig. 723. A pansporoblast with eight sporoblasts.

Fig. 724. A pansporoblast containing young spores.

Fig. 725. A pansporoblast with mature spores in fresh condition.

Fig. 726. Fresh spores.

Fig. 727. Stained spores.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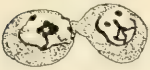
KUDO A STUDY OF THE MICROSPORIDIA PLATE XXIII

PLATE XXIV

EXPLANATION OF PLATE XXIV

Figs. 728 to 730. *Nosema anophelis*. Original.
Fig. 728. A schizont undergoing binary fission. ×3200.
Fig. 729. Fresh spores. ×2400.
Fig. 730. Stained spore. ×3200.
Figs. 731 to 737. *Thelohania obesa*. Original ×3200.
Figs. 731 to 736. Stages in sporogony.
Fig. 737. Stained spores.
Figs. 738 to 742. *Thelohania pyriformis*. Original.
Figs. 738 and 739. Tetranucleated and octonucleated sporonts in smears. ×3200.
Fig. 740. Fresh spores. ×2360.
Fig. 741. Stained spores. ×3200.
Fig. 742. A spore with extruded filament. ×800.
Figs. 743 and 744. *Thelohania rotunda*. Original ×3200.
Fig. 743. A mature octosporous pansporoblast in a smear.
Fig. 744. An isolated spore.
Figs. 745 to 748. *Thelohania minuta*. Original.
Fig. 745. A mature octosporous pansporoblast. ×3200.
Fig. 746. Stained spores. ×3200.
Fig. 747. Fresh spores. ×2360.
Fig. 748. End view of a spore in fresh state. ×2360.
Figs. 749 and 750. *Thelohania opacita*. Original ×3200.
Fig. 749. A compressed spore showing the filament extruding from its side.
Fig. 750. A moderately compressed spore showing its sutural line on the membrane.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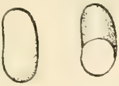
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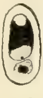
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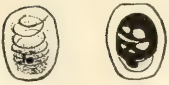
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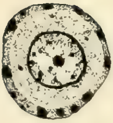
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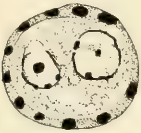
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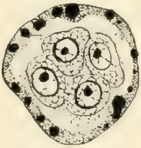
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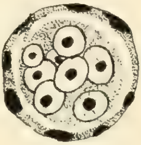
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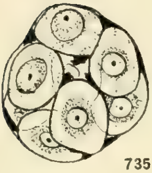
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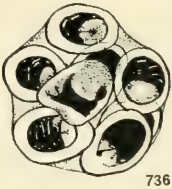
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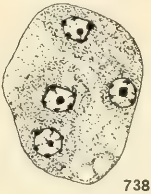
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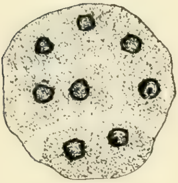
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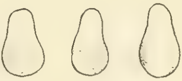
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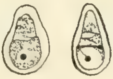
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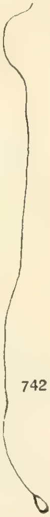
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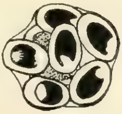
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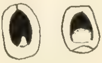
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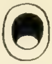
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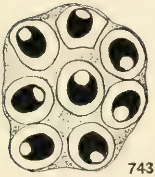
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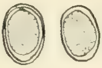
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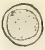
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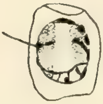
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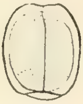
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PLATE XXV

EXPLANATION OF PLATE XXV

Fig. 751. The posterior portion of *Gammarus pulex* infected by *Thelohania mülleri*. After Léger and Hesse. ×5.

Fig. 752. The posterior portion of *Gammarus pulex* infected by *T. giraudi*. After Léger and Hesse. ×5.

Fig. 753. Three segments of *Diaptomus castor* infected by *Gurleya richardi*. After Cépède. ×50.

Fig. 754. *Diaptomus gracilis* infected by Genus inc. *colorata*. After Fritsch.

Figs. 755 and 756. Two views of a larva of *Corethra plumicornis* infected by *T. corethrae*. After Schuberg and Rodriguez.

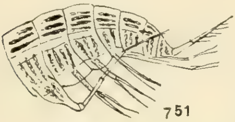
Fig. 757. A larva of *Bombyx mori* suffering from a heavy infection by *Nosema bombycis*. Original. Natural size.

Fig. 758. The central nervous system of *Lophius piscatoris* infected by *Nosema lophii* After Doflein.

Fig. 759. A part of the intestine of *Pleuronectes platessa* showing infection by *Glugea stephani*. After Woodcock.

Fig. 760. A portion of the testis of *Barbus barbus* infected by *Plistophora longifilis*. After Schuberg. ×1.5.

Fig. 761. A cross-section of *Gasterosteus aculeatus*, showing two large masses of *Glugea anomala*. After Thélohan.



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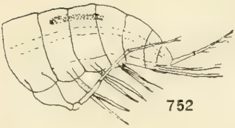

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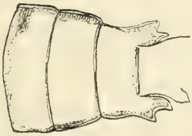

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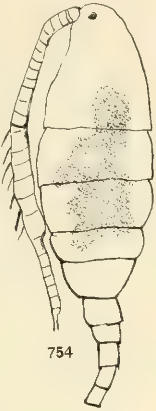

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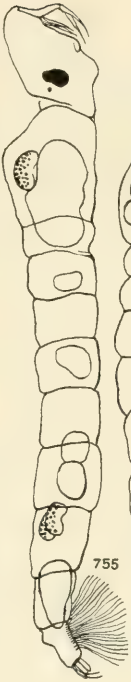

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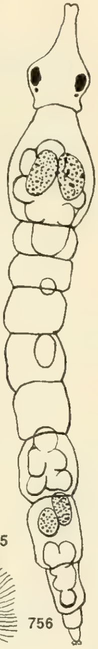

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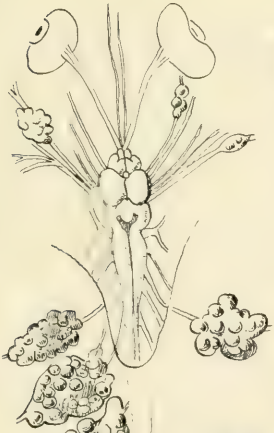

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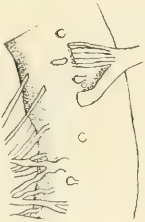

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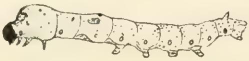

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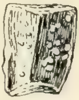

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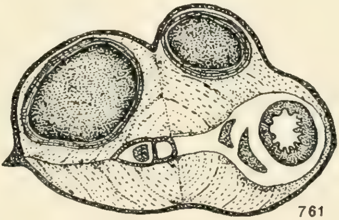

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PLATE XXVI

EXPLANATION OF PLATE XXVI

Fig. 762. The gills of *Gadus aeglefinis* infected by *Nosema branchiale*. After Nemeczek. Natural size.

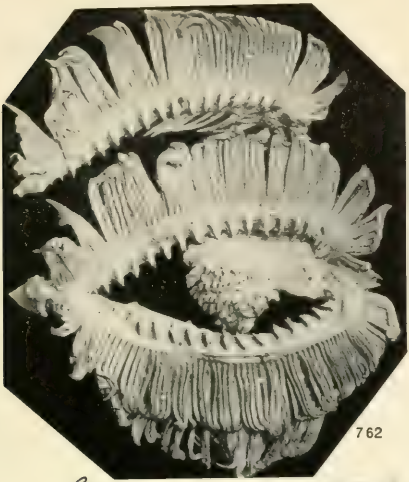
Fig. 763. The dorsal view of a larval *Culex testaceus* infected by *Thelohania opacita*. After Kudo. ×11.

Fig. 764. A larva of *Anopheles crucians* infected by *T. legeri*. Original ×5.

Fig. 765. A simulium larva infected by *T. multispora*. After Strickland. ×12.

Fig. 766. A section through the abdomen of a Simulium larva infected by *T. fibrata*. After Strickland. ×12.

Fig. 767. A smelt infected by *Glugea hertwigi*. After Schrader.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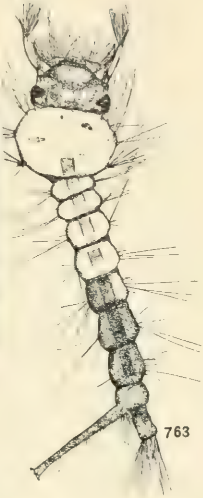
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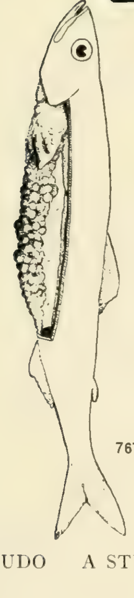
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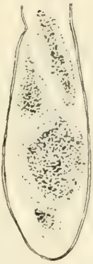
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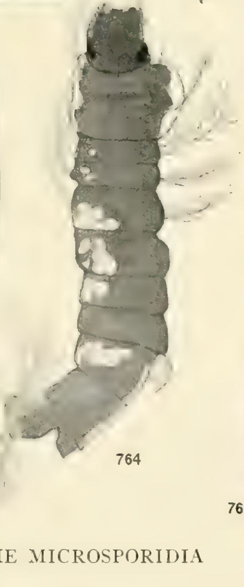
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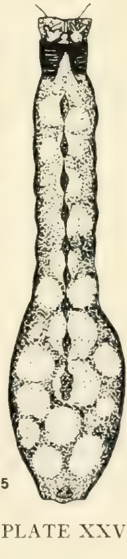
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PLATE XXVII

EXPLANATION OF PLATE XXVII

Fig. 768. A portion of section through canaliculus seminalis of the testis of *Barbus barbus* infected by *Plistophora longifilis*. After Schuberg.

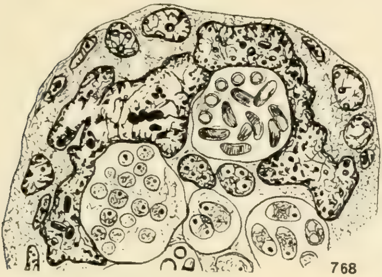
Figs. 769 and 770. Normal and hypertrophied nuclei of the fat body of *Culex pipiens*, the latter affected by *Stempellia magna*. After Kudo. ×1500.

Figs. 771 to 775. Hypertrophied nuclei of the adipose tissue cells of *Simulium reptans* infected by *Thelohania varians*. After Debaisieux. ×2250.

Figs. 772 to 774. Normal and hypertrophied nuclei of the adipose tissue cells of Baetis nymph infected by *Nosema baetis*. After Kudo. ×1500.

Fig. 776. Part of an epithelial cell of the mid-gut of larval *Ptychoptera contaminata* infected by *Gurleya francottei*. After Léger and Duboscq.

Figs. 777 to 779. A normal (Fig. 777) and two hypertrophied nuclei of the connective tissue cells of *Chaetogaster diaphanus* found in the "cyst" of *T. chaetogastris*. After Schröder.



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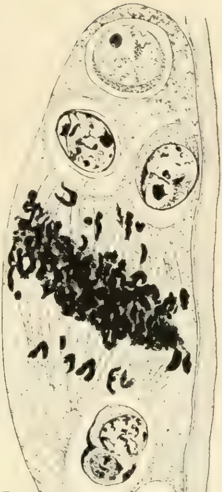

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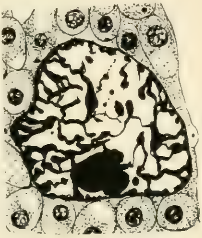

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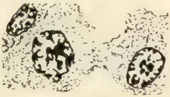

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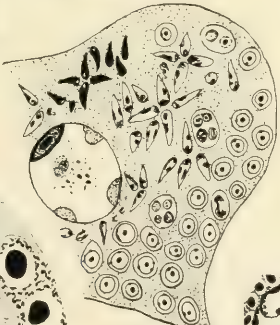

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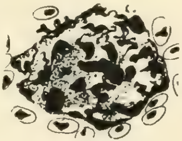

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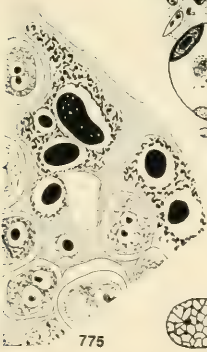

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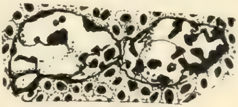

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ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WITH 7 PLATES AND 15 TABLES

BY

ASA ORRIN WEESE

Contributions from the
Zoological Laboratory of the University of Illinois
No. 250

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INTRODUCTION

Quantitative methods have been applied but little to the study of animal communities on land, although the quantitative study of life in the sea has, particularly in the hands of the Danish oceanographers, yielded rich results. Among the most important contributions dealing with animal populations on land are the papers of McAtee (1907), Beebe (1916) and Wolcott (1918), all of whom studied definite limited areas. Of these papers, only that of Wolcott dealt in any way with the successional phases of the problem. The problems of stratification and migration were attacked by Sanders and Shelford (1922) with reference to a pine-dune community. This study covered a period of about two months.

The writer has attempted to investigate quantitatively the animal population of the elm-maple association as represented in a forest in the "savanna" region of Illinois, during an entire year, with supplementary observations covering a greater period of time. During this time a series of meteorological observations was carried out covering as many as possible of the environmental conditions of the habitat, and an attempt was made to correlate the fluctuations in the animal population with changes in environmental conditions. A characterization of the seasonal and stratal communities within the association became possible on the basis of the information gained by the above investigations. Correlated experiments on the reactions of animals in gradients of the environmental factors thought likely to be effective in stratification, and the effect of environmental factors on the rate of development of characteristic species were also carried out.

The present paper gives the results of the above investigations in the following order: (a) a study of the animal population as a whole by the method of random sampling, (b) a study of the stratal and seasonal abundance of certain species prominent in the population, with especial reference to migration, (c) a study and characterization of the communities of the forest, with especial reference to the seasonal succession, (d) the reactions of animals in gradients, (e) experiments on the effect of environmental factors on the rate of development of a spider, *Acrosoma rugosa* and its parasite, *Arachnophaga picea* and (f) a record of environmental conditions in the habitat.

THE HABITAT

The area studied is some sixty acres in extent, located about five miles northeast of the campus of the University of Illinois, and known as "University Woods," the botanical, zoological and entomological reserve of the University of Illinois. The 700-foot and 690-foot contour lines pass through the tract. The drainage is poor, so that in wet seasons the soil becomes saturated with moisture, and water may stand for some time in the Spring in depressions even in the higher parts of the woods. The soil is yellow-gray silt loam, an upland timber soil whose limits are but little beyond the present border of the woods to the north and east. (Hopkins, et. al. 1918). McDougall (1922) has fully described the plant community of this forest. The higher parts are well wooded with a stand composed almost purely of maple (*Acer saccharum*) while a mixed stand of maple and elm (*Ulmus americana*) occupies the intermediate levels and elm is the dominant tree on the lower ground. Other prominent trees are, in order of abundance, *Fraxinus americana*, *F. quadrangulata*, *Tilia americana* and *Carpinus caroliniana*. Many others, such as *Aesculus glabra*, *Quercus rubra*, etc., are present in smaller numbers, and in the more open situations there is a considerable growth of underbrush, largely *Benzoin melissaefolium* and *Asimina triloba*, with seedlings of the various trees comprising the forest.

The tract was for some time rather heavily pastured and some cutting of hardwoods (especially *Juglans nigra*) of which the stumps remain, had been done, but since its acquirement by the University of Illinois some five years previous to the present study, it has been left practically undisturbed. Accordingly reproduction of the woody plants is taking place to a considerable degree.

This area is one of the few remaining bits of the woodland which originally extended into the prairie of central Illinois along the Vermillion River and its tributaries, and like all other similar tracts it has been profoundly modified by the activities of civilized man. The first modification was, doubtless, the elimination of the larger mammals frequenting the deciduous forest under primitive conditions. The clearing for agricultural purposes of the greater part of the former wooded area limited the migration of species restricted to the dense woods, so that when a species lost its hold, for some reason, on a particular area, it was not replaced even on the return of favorable conditions. It is probable that the period of heavy grazing drove from this particular area many forms which have been unable to return even though the conditions are now favorable for them, because of the isolation of the tract and the absence of suitable migration paths. No characteristic mammals, with the exception of the wood mouse (*Peromyscus leucopus noveboracensis* (Fischer), are present in any number. Even squirrels of all kinds are rare.

The utilization of the neighboring lands for agriculture has led to the incurrence of many introduced forms, especially among those which may be classed as garden and orchard pests. Although, as will be seen above, the conditions studied here do not represent exactly the primitive conditions of the Illinois elm-maple forest, they represent the closest approximation available for study at the present time.

In order to determine the physical conditions prevailing in the habitat under consideration and to determine the character of the climatic rhythm to which the animals living there are subject, it was necessary that an extensive series of meteorological observations* be made. The observations were made at stations as near as possible to the places at which the population samples were obtained and covered the period from July 1, 1921, to July 1, 1922. Salient features are illustrated in figures 6 and 7. A summary follows:

During the summer months the mean daily temperature in the elm-maple forest increases upward from the ground level at the rate of approximately 0.35° C. per ten meters, and relative humidity decreases 3.5 per cent in the same distance. These gradients may be absent or reversed during early morning hours, and during the colder months of the year (Fig. 2, Tables A, B, C, D, E).

The evaporating power of the air is least at ground level, averaging for the period of observation 5.96cc. mean daily evaporation from a standard porous cup atmometer, and increases upward, at first rapidly and then more slowly to a maximum of 15.71cc. at the level of the tops of the trees (Fig. 2, Table F).

The intensity of light in the forest increases rapidly from the ground level up. At noon of a sunny day the intensity at ground level under herbage is about 0.35 per cent of full sunlight, while that at 1.25 m. is about three times as great (Table G).

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* A detailed account of methods and results is given in the summary of meteorological observations.

THE ANIMAL POPULATION

Throughout the period of study random samples were taken periodically from the shrub, herb, and ground strata of the forest. Obvious difficulties prevented random sampling from the tree stratum. Because of the leaves on the surface of the soil, the ground stratum was divided into two parts, referred to as the leaf and soil strata. Of the latter only the upper 10 cm were considered.

The unit sample from the soil and leaf strata was taken from an area 2 ft. (0.61 m) square. Leaves and other debris from this area were gathered and placed in fine wire screen boxes, the contents of which were sorted in the laboratory after the animals had been quieted by the use of ether. After the leaves had been removed the soil of the bared area was carefully gone over to a depth of 10 cm and all animals obtained placed in vials for further assortment.

The unit sample from the herb and shrub strata was that obtained by ten short sweeps through the vegetation with an insect net whose subcircular opening averaged 30 cm in diameter. The contents of the net were transferred to screen cylinders and taken to the laboratory for further examination.

The method of collection was probably most efficient in the leaf stratum, the soil stratum and the herb and shrub strata following in the order named. Assuming the average depth of the herb stratum to be 0.5 m and the average length of the sweep of the net to be 1.0 m, calculation shows that ten such sweeps include approximately the volume of vegetation above the unit area. The foliage of the shrub stratum may also be assumed to be of approximately the same depth, although the relative efficiency of the sampling method is somewhat less in this stratum. All collections were made near the stations from which the meteorological data were obtained, and samples at the various levels were, in general, taken within a few minutes. In the tables and the text all collections are dated according to the last day of the week in which the collection was made, reckoning from Monday to Monday. This day was chosen because the hygrograph and thermograph sheets were changed at this time, and means of climatic factors could be computed most easily sheet by sheet.

The numerical data obtained from samples taken in the manner described above are given in Table H and the relations are illustrated graphically in Figure 1, A. In the curve representing the total population in all strata, we find a drop in the fourth week (the week of July 25) corresponding to an increase in the evaporating power of the air (See Figure 2, G-L). At the beginning of the period of study, the woods were dry, due to a long period of heat and drouth during June, and the animal population was already small. Sanders and Shelford (1922) found an increase of population with drouth and high temperature in a pine-dune community. With the increase in moisture content of the air (and of the soil-leaf strata) during the following weeks, there was a marked increase in the size of the collections, and the great increase culminating in the high maximum during the fourteenth week (ending October 3) might seem to be due to this cause. A gradual decline in temperature (Figure 2, C) had been going on, however, with a considerable increase in mean variability (Figure 2, E) due to lower temperatures at night, so that, while the initial increase was due to a return to moisture conditions more nearly optimum for the species concerned, the sudden and great increase was due almost entirely to the autumnal migration toward place of hibernation. An analysis showed that the maximum was caused almost entirely by a few genera of beetles and two genera of Cicadellidae, chiefly hibernating species in course of migration from the forest margin to the leaf stratum of the more protected portion of the woods. Following the maximum, the fall was rapid, as temperatures continued to fall (the first heavy frost occurred the night of October 4), stimulating the insects to seek more complete shelter. The peaks in the totals curve during the winter months after November 13, the date of the first ice formation, were caused by samples taken on warm days when insects partially emerged from hibernation, or at least, approached near enough to the surface of the soil or leaf strata to be collected. The striking population increases of spring began about April 10, reaching high maxima on May 1 and May 8. The great numbers on these dates were principally a forest margin beetle, *Epitrix brevis*, and a bug *Corythucha aesculi* which inhabits the tree stratum. As these insects passed on to their summer habitats, the population of the lower strata again decreased sharply. The determining factors of the sudden appearance of great numbers of these insects could not be definitely determined. Increase of temperature and greater variability, especially in the ground strata were probably of great importance.

The largest numbers, during the summer months, were found in the leaf and herb strata, and, for the most part, the curves (Fig. 1, B D) representing the population of these two layers varied together. Both showed marked minima during the excessively dry week of July 25, and both rose as the weather became more favorable. The leaf stratum as well as the herb stratum exhibited the marked maximum of October 3, due to the influx of hibernating insects. The low point in the leaf stratum curve for October 17 may have been due to the chance selection of a poorly inhabited area. At any rate, after the fall in the herb stratum curve, there was a second maximum, due evidently to the downward migration of the insects which were, the week before, in the herb stratum. After this date, the decline of the herb stratum curve was very rapid, and the course of the totals curve was almost entirely governed by that of the leaf stratum. The large numbers found in certain of the late winter collections were partly due to the appearance of larvae of forms passing the winter in this state. In the spring the population of the leaf stratum remained greater than that of the herb stratum until after April 10, which marked the first appearance of abundant vernal herbage. The maximum at the latter level was reached May 8, the most abundant insect being *Epitrix brevis.* An equally rapid fall in numbers followed the rapid migration of this species.

The shrub stratum (Fig. 1,C) showed, in general, variations in population paralleling those of the strata below. It is of interest, however, to note that the autumn maximum in this stratum came a week earlier than that of the herb stratum. The collection of this date, also, was the only one showing a larger population in the shrub than in the herb stratum. This relation was due to the fact that the insects of the forest margin first migrated inward at the level of their summer occurrence, later migrating downward for hibernation. The spring maximum of the shrub stratum, due largely to the upward migration of *Corythucha aesculi*, occurred on May 1.

The most striking phenomena of the entire period covered by the collections were the hibernating reaction of the autumn, involving a migration inward from the forest margin and downward to the forest floor, and the migration in the opposite sense in the spring. The principal inciting factors of the former seemed to be the fall in temperature and the great daily range of temperature of the early autumnal period. The latter was likewise a response to the changing temperature conditions of the forest, supplemented, perhaps, by changing moisture and light conditions. The fact that many species react alike and at the same time to the same stimulus or combination of stimuli shows a great degree of similar adjustment to the climatic rhythm of the temperate savanna on the part of the characteristic insects of the region.

DOMINANT SPECIES

Certain species which were present in the samples over a considerable period of time, or abundantly for a shorter period, were chosen for further study from the standpoint of seasonal succession. The animals chosen were spiders, Hemiptera and Coleoptera, because of the greater abundance and more conspicuous character of these groups. Data as to seasonal succession and seasonal variations in abundance of these species may be best visualized by reference to figures 3, 4 and 5.

Migratory Forms

Notoxus monodon Fab. [Figure 3B]

This beetle (*Anthicidae*) was first taken from the herb stratum August 29, the maximum at this level being reached during the week of October 3. It is a forest margin species hibernating in the forest, and its early appearance on forest herbage represents the first step in this migration. Very large numbers were found among leaves on the ground October 31, and during the entire winter, on warm days, especially, considerable numbers were taken from this stratum. A second maximum corresponding to the spring migration occurred on April 3, and on April 10 small numbers were again found in the herb stratum.

Telephanus velox Hald. [Figure 3E]

This beetle (*Cucujidae*) was found on herbage October 3, and thereafter throughout the winter in the leaf stratum. The autumn maximum was on November 7 and the spring maximum on April 10.

Phalacrus politus Melsh. [Figure 3C]

Included in the chart are other Phalacridae as well as *Phalacrus politus*, to which species most of those taken belonged. They were among the most abundant beetles found after September 26, when they were observed in the samples from all three strata. The numbers from the shrub stratum were small, however, and none were taken from this level after October 31. The maximum in the herb stratum came October 3, and on this date, also, was the first maximum for this family in the leaf stratum. A second maximum appeared on November 7. The week ending November 7 was characterized by three fairly heavy frosts, and a marked general fall of temperature. The first maximum, on October 3, followed a period of falling temperature, and great variation between night and day, without any actual frost, while the maximum in the leaf stratum followed a similar period with considerably lower temperature. *P. politus* is a forest margin species and its presence in the denser forest was an indication of a hibernation migration. The species was found throughout the winter in the leaf stratum. In the spring these beetles began to be taken in considerable numbers from this stratum on March 27, the maximum here being reached May 8, and in the upper strata on May 15. Thus the vernal migration is shown to follow the route taken in the autumn, in inverse order.

Glyptina spuria Lec. [Figure 3F]

This Chrysomelid was found in the shrub stratum from September 5 to November 7, and after that date in the leaf stratum. A few individuals were also swept from the herb stratum on November 21, which was a comparatively warm and sunny day. The summer habitat of this species is the forest border and roadside, and its appearance in the forest collections is due to its hibernating reactions. Individuals were found throughout the winter, especially on warm days. In the spring very large numbers were found in the shrub and herb strata. They were found first in the herb stratum on March 13 and in the shrub stratum on April 24, but the maximum for both strata occurred on May 8.

Epitrix brevis Schw. [Figure 3G]

Enormous numbers of *E. brevis* (Chrysomelidae) were swept from the herb stratum October 3, the first individuals being taken from this level on September 19, and the last, October 17. A few were swept from the shrub stratum during the week of September 26, and a few hibernating individuals were obtained January 16 and 23. The sudden appearance of this beetle represents a migration, *en masse*, in preparation for hibernation. The small numbers taken during the winter are due, evidently, to deep hibernation. Beetles of this size and color would also be difficult to distinguish in soil, so that perhaps some may have escaped notice. In the spring this species appeared in the shrub stratum on April 10, a very high maximum being reached on May 8, after which the decline was rapid. No great numbers appeared at any time in the herb stratum. Evidently no halt is made by *E. brevis* in the lower stratum on the way either to or from the place of hibernation.

Epitrix fuscula Crot. [Figure 3H]

While not found in as large numbers as the other member of the genus, this little beetle was fairly abundant after September 26. None were found elsewhere than in the leaf stratum where hibernation took place. It was found in much larger numbers through the winter than *E. brevis*. This, also, is a forest margin species, feeding principally on plants of the family Solanaceae, near the roots of which the eggs are laid in the spring. According to Somes (1916) there are three broods per year in Missouri.

Chaetocnema confinis Crot. [Figure 3I]

This forest border chrysomelid was first taken from the shrub stratum September 19, becoming abundant on September 26. Thereafter the numbers appearing in the samples decreased. One individual was taken from the leaf stratum November 14, and one from the herb stratum May 8. It is probable that the species hibernates in such situations as not to be found ordinarily by the methods of collection used.

Phyllotreta sinuata Steph. [Figure 3J]

This chrysomelid of the forest border and meadow was found in small numbers on July 18 in the leaf and soil strata, but the real migration did not begin until October 3, culminating in a maximum on October 17. Hibernating individuals were noted in the collections throughout the winter, the number observed increasing with the first warm days of the season.

The principal migration of this beetle took place only after several rather severe frosts.

Longitarsus melanurus Melsh. [Figure 4K]

Although taken from the herb stratum only, the frequency polygon of this beetle (Chrysomelidae) is typically like those of other beetles coming to the forest for hibernation, and it is probable that hibernation took place in situations from which collections were not made. This species is found during the summer in the rank herbage along roadsides and in other similar situations.

Diabrotica vittata Herbst. [Figure 3A]

The striped cucumber beetle is a forest margin beetle which has become a pest by invasion of cultivated fields. Autumn finds many individuals in the forest in preparation for hibernation. The first large collection was from the leaf stratum on September 12. On sunny days later it was swept from shrub and herb strata. Remains of dead beetles found during the winter indicated that the number completing hibernation is probably relatively small. Probably only those in the most protected situations are able to survive. In the spring it was found first in the leaf stratum, then in the herb and shrub strata. The spring maximum was April 24. Mating takes place in early spring on Crataegus and Prunus, typical forest margin trees.

Phytonomus nigrirostris Fab. [Figure 3D]

The lesser clover-leaf weevil seeks its hibernating quarters very early in the season. According to Mr. Faustino Otanes, who made systematic collections on alternate days from a clover field on the experimental farm of the University of Illinois, very few were found there after July 7. They were found, in small numbers, in the woods in the herb and leaf strata from the beginning of the series of collections. None were swept from herbage after September 26. Maximum collections were made from the leaf stratum on September 12 and October 31. The former date followed a week of declining temperatures, which evidently caused greater numbers to seek the protection of the leaf stratum, while the latter date was in a period of rising temperature. Throughout the winter these beetles were found in the leaf stratum in increased numbers on warm, sunny days, indicating a vertical migration according to temperature. *Phytonomus nigrirostris* is an introduced species, coming from Europe, and was first collected in Illinois by Mr. W. P. Flint in 1919.

Empoasca viridescens Walsh. [Figure 5Y]

The leaf-hopper named above was not noted at all until September 19, and appeared in very large numbers on September 26, principally in the herb stratum, but to a lesser extent in the shrub stratum. It was swept from these strata in very large numbers during a period of about six weeks, the largest numbers occurring on September 26 and October 31. The intervening period was marked by low temperatures, which probably caused the insects to retire to more sheltered locations. Leaf-hoppers are also known to migrate downward to the base of herbage during dry weather, so that the numbers obtained by random sweeping are smaller under such weather conditions. After December 1, no Cicadellidae were found elsewhere than in the leaf stratum and in the soil below. They were more abundant in the collections on warm days when they could be seen moving about on the surface of the leaf layer. They emerge very early in the spring, as soon as food plants are available.

Erythroneura obliqua Say (and varieties). [Figure 5Z]

The frequency curve for this leaf-hopper, in almost all points, is similar to that for the form just discussed. The first maximum occurred a week later and the second maximum was relatively higher. Only a few were found in the herb and shrub strata, and practically all were taken from the leaf stratum. Greater numbers of this species were taken during the winter than of the preceding.

Corimelaena pulicaria (Germ.). [Figure 4M]

As this form (Cydnidae) is of little economic importance, not much is known of its life history. However, it is an insect of the forest border, migrating inward to the more densely wooded areas for hibernation in the autumn, and emerging again in the spring. The frequency polygon is similar in detail to that of *Phalacrus politus* (Fig. 3C).

Lygus pratensis (L.) the tarnished plant bug, and *Blissus leucopterus* (Say), the chinch bug (Figure 4N and O), are too well known to require comment. The latter shows well the two periods of abundance although they are somewhat closer together than for most of the other insects studied. Lygus is a forest border form feeding on the forest border trees as well as on herbage. The latter is primarily a grass feeder.

Corythucha aesculi O. & D. [Figure 4L]

This lace-wing (Tingidae) is included among the migratory forms, although the migration in this case is vertical instead of horizontal. It inhabits the tree stratum during the summer. It began to appear in very large numbers in the shrub stratum on April 24, reaching a maximum a week later. This coincided with the first abundance of vegetation in this stratum, and with the development of the tree foliage these insects spread to the higher levels, their abundance at the shrub level rapidly falling off. A few individuals had been found, throughout the autumn and winter, in hibernation in the leaf and ground strata. Corythucha winters, generally, in the adult form, depositing the eggs in early spring, but may also winter in the egg stage.

The large numbers of beetles and other insects in the late summer and autumn collections were mostly due, not to species feeding and breeding in the forest, but to forest border and prairie species which migrate forestward in preparation for hibernation. Many of the leaf-beetles belonging to the forest proper also hibernate, but in no case were the numbers taken in random sampling sufficiently large to warrant the construction of frequency polygons illustrating their relative abundance. The question of how far into a larger forest species such as these would penetrate for hibernation is unanswered. It is probable that the distance would vary with different species, but the tract of woodland studied was too small to afford a solution of this problem.

In most of the species considered the stimulus initiating migration was apparently the gradual increase, in early autumn, in the daily range of temperature, caused by lower night temperatures. Some species required the additional stimulus of a frost of greater or less intensity, while others which appeared in the forest very early did not require this stimulus. The disappearance or maturation of the food plant may have been a factor in some cases. The majority of the insects appeared in maximum numbers between September 26 and October 3, a period characterized by falling temperatures and a marked drop in the night minimum.

In the forest, the insects appeared first in large numbers in the stratum corresponding to that in which the summer portion of their life history was spent. Insects whose principal summer food plant is in the high forest margin, first appeared in the shrub stratum. Those whose summer habitat is the low forest margin or meadow, first appeared in the herb stratum. A downward migration followed under the stimulus of an additional fall in temperature. Insects from the shrub stratum, in most cases, spent a short period of time in the intermediate herb stratum before seeking the final place of hibernation in the leaf or soil strata. Warm days reversed the course of migration, and even in winter, high temperatures brought hibernating insects nearer to the surface, so that they were taken in greater numbers. Many species showed spring maxima also, due to the breaking up of hibernation. These occurred uniformly shortly after the first definite spring rise in temperature and the appearance of vernal herbage.

Non-Migratory Forms

Uloborus americanus Walck. [Figure 42]

The frequency polygon illustrating the occurrence of this spider is in two parts, the heavy line representing the adults and the light line the juveniles. Adults, at first more abundant than the young, disappeared entirely after October 17. The first young were taken August 22, and increased rapidly in numbers until October 17 after which they, also, disappeared from the collections. This species hibernates when half-grown, reaching maturity in early summer. The young emerge from the egg in the latter part of July.

Dictyna [Figure 4S]

Most of the young spiders of this genus probably belonged to the species *volupis*, but were too young for positive identification. The web of this spider is made in the hollow of a slightly rolled leaf, where the eggs are deposited in July. Young and half-grown individuals were abundant August 29, and the largest numbers for the season were taken on October 3. The numbers then declined, and after October 24 none were found except in hibernation in the leaf stratum. As was the case with the insects these spiders were also found on the warm winter days.

Tetragnatha [Figure 5X]

Adults and young of this genus were not found together, as the former appeared in the collections only in the very early part of the season, none at all being found after the second week in July. The young appeared first on October 24, and first became abundant on November 21. The maximum occurred on December 19. These spiders were found almost invariably in the shrub and herb strata, very few being found in the lower strata even during the very cold weather. Hibernation, in Tetragnatha, simply means seeking a somewhat sheltered place in a crevice in the bark or some similar location, for a few hours or days when weather conditions are very unfavorable. As soon as the air becomes slightly warmer, the spiders emerge and again become active.

Epeira gibberosa Hentz. [Figure 5W]

This widely distributed species was the most abundant spider during the later summer months, reaching a maximum during the week of August 29. None were found after November 7. This species does not hibernate in the adult stage, but eggs are deposited during the early autumn.

Cocoons of this species are formed from the small folded leaves of shrubs, the petioles of which are reenforced by strands of silk and which are lined and fastened together at the edges with silk. These cocoons became very numerous after October 15. One other unknown species forms similar cocoons, generally in larger leaves and at a greater height from the ground. The eggs hatch within a month or so after deposition, and the young pass the winter in the cocoon, to emerge in the spring. Experiments indicate that there is a period of dormancy after the emergence from the egg of the young spider, and that, although favorable conditions may bring about emergence from the cocoon of spiders which have not been subjected to prolonged low temperatures, those which have experienced such temperatures emerge more quickly upon the return of favorable conditions.

The cocoons are very heavily parasitized by a hymenopterous insect, *Arachnophaga picea* Riley. Of 52 cocoons examined on January 9, 22 were found to have been parasitized by some organism which had already emerged, and four were found to contain hymenopterous larvae, of which there were, in each case, two. Of 350 cocoons used in experiments, 47 produced from one to five individuals of *A. picea.* In the greater number of cases, there were two insects, one male and one female, in each cocoon. Single individuals of each of two other species also emerged from other cocoons of the same lot.

Acrosoma rugosa Hentz. [Figure 5V]

This conspicuous spider was very much in evidence during the late summer, particularly in more shady situations. Only a single male was taken on July 11. While the females must have been as abundant at this time as later they did not reach their greatest prominence until the end of August. After this the numbers decreased rapidly, and the last one was taken October 10. This species does not hibernate in the adult stage, but passes the winter in the egg.

Xysticus elegans Keys. [Figure 4T]

Adults were found in small numbers during the earlier part of the season, the last on October 10. The young were very numerous throughout the summer and the greater part of the winter. Maxima were evident on August 1 and October 17. After the latter date none were found in the herb stratum whence the majority were taken during the summer. A large number also appeared from hibernation on February 20 and 27, after the ground had been warmed by a series of warm days.

Anyphaena rubra Emer. [Figure 4R]

Spiders presumably of this species, mostly immature, were taken throughout the season, the maximum number being recorded for October 17, mostly in the herb and shrub strata prior to November 14, and in the leaf stratum after that date. Adults mature in early summer and hibernation takes place in the half-grown state. Large numbers were taken from rolled leaves during October. An increase in the number of individuals in the collections was noted during the warm weather of the latter part of February.

Dendryphantes aestivalis Emer. [Figure 4P]

The frequency polygon for this species is almost exactly the same, except for a slightly earlier appearance of the young, as that of Uloborus previously discussed. In the latter part of the season, many young were found in rolled leaves, evidently in preparation for hibernation.

Linyphia phrygiana Koch. [Figure 5U]

Young of this species were abundant during the entire summer and autumn. The last to be swept from the shrub stratum were observed on

November 28. A hibernating individual was found in the upper soil layer on December 12, and specimens were again found on low herbage as early as January 9.

The spiders listed above, unlike the insects previously mentioned, are permanent residents of the forest (some are found also outside the forest), instead of forms coming to the forest mainly for purposes of hibernation. In some cases the frequency polygons expressing the relative abundance of certain species of spiders are quite similar to those of the beetles, with the maxima somewhat less abrupt. The rise here, however, was not due to an inward migration of the species but to the growth of the young and, in some cases, to their migration through a relatively short distance to the location in which they were taken. The species having this form of curve are those which spend the hibernation period as approximately half-grown juveniles, and mature early the next year. *Dendryphantes aestivalis*, *Uloborus americanus*, *Anyphaena rubra*, *Dictyna volupis* and *Xysticus elegans* constitute a unit in so far as adjustment to the climatic rhythm of the temperate deciduous forest is concerned, as the differences in their annual cycles are small and relatively unimportant. Another less homogeneous group is exemplified by *Acrosoma rugosa* and *Epeira gibberosa*, which ordinarily spend the winter within the egg case. In these the life cycle is much the same except that the period of dormancy comes earlier in the life of the individual, and the period of maturity comes later in the summer. Tetragnatha and Linyphia belong, loosely, to a third group, differing mainly in the degree of activity during the winter. Tetragnatha seems to show a more rapid rate of development, or perhaps, a more continuous development through the winter, as indicated by the earlier maturity and the later appearance of the young.

ANIMAL COMMUNITIES

The animal communities of a given region cannot be considered, logically, separately from the plant communities. In fact, the division into animal and plant communities is often an entirely arbitrary one, and the dominants of a given community may be plants or animals or partially one and partially the other. It is customary to speak of the larger terrestrial communities, at least, in the terms of plant dominants, to the exclusion of animal dominants. This is partially because of the more obvious nature of the differences in vegetation-form of the climax formations, and partially due to the fact that the plant dominants of the biotic community often exhibit actually a greater degree of dominance than the animals. This is probably true to a much greater degree in forest communities than in those characterized by lower types of vegetation-forms.

In a savanna such as that studied in the present instance there are two distinct types of plant communities, that of the woodland and that of the grassland. The dominant woodland species are in the present instance, *Ulmus americana* and *Acer saccharum*, both characteristic dominants of the Eastern Deciduous Forest Formation. The dominant of the grassland community is, according to Sampson, *Andropogon furcatus*. Clements considers this community as an associes leading here to a deciduous forest climax, and farther west to a true prairie climax (Stipa-Koeleria Association). The plant societies of the Andropogon associes are, for the most part, those of the Stipa-Koeleria association, although there are a few invaders from woodland and thicket. It will be noted that Clements does not recognize a savanna formation as such, but considers the woodland components as approaching the climax of the deciduous forest formation, and the grassland as an associes of a subclimax whose final goal is also (here at least) the deciduous forest. No attempt has been made, in this paper, to study the animal components of the grassland community except in so far as these animals appear as seasonal migrants in the forest. Particular stress has been placed upon the animal components of the forest community.

As stated above, the plant dominants of the woodland community are *Ulmus americana* and *Acer saccharum*. The community may be spoken of as an elm-maple association, whether we consider it as a part of a savanna formation or as a unit in the temperate deciduous forest. This association is more or less distinctly divided into the maple and the elm consociations. The subdominants of the shrub stratum are *Asimina triloba* and *Benzoin* *mellissaefolium*. Seasonal societies are in the main absent at this level. In the herb stratum, however, seasonal phases are distinctly marked. McDougall distinguishes the following subdominants of the respective societies:

Prevernal societies
    Claytonia virginica
    Isopyrum biternatum
    Collinsia verna
    Dicentra cucullaria
    D. canadense
    Phlox divaricata
    Geranium maculatum
    Floerkia proserpinacoides

Vernal societies
    Hydrophyllum appendiculatum
    H. canadense

Aestival societies
    Laportea canadensis
    Impatiens biflora
    I. pallida

Serotinal and Autumnal societies
    Campanula americana
    Eupatorium urticaefolium
        and other compositae.

The ground strata have not been studied from the botanical standpoint.

DOMINANTS AND SUB-DOMINANTS

The seasonal distribution of some of the principal components of the animal community has been indicated in the preceding section. An attempt will now be made to characterize the seasonal animal societies which correspond more or less in duration to the plant societies just mentioned.

The prevernal period, beginning about the first of March, is characterized by rapidly rising temperatures of both air and soil accompanied by a great deal of rainfall, with a resulting high moisture content of air and soil. As most of the forms present at this time are in or near the soil stratum, the changes in conditions at this level are of the greatest importance.

The animal society of the prevernal period is very inconspicuous as but few of the insects have risen far above their places of hibernation. It is difficult to decide as to dominance at this time but *Phalacrus politus*, *Epurea rufa*, *Epitrix brevis* and *Notoxus monodon*, all hibernating beetles, seem to be most numerous. The spider *Tetragnatha laboriosa* is the only prominent organism in the herb and shrub strata. *Anyphaena rubra* and *Lycosa rubicunda* (spiders) are found in the leaf stratum with the beetles mentioned above. The spiders are the only subdominants not migratory in character.

The vernal period may be said to begin with the appearance of abundant vernal herbage, about the first of April. There is, at this time, a well-developed plant society of the herb stratum and the leaves are appearing in the shrub stratum, and, somewhat less conspicuously, in the tree stratum. This is a period of conspicuously large population due to the upward and outward migration of insects of the tree stratum and of the forest edge.

The dominants of the vernal society are, if migratory forms may be considered, *Epitrix brevis*, *Glyptina spuria* and *Diabrotica vittata* in the herb stratum, and *Diabrotica vittata* and *Corythucha aesculi* in the shrub stratum. Other species of prominence are *Phalacrus politus* in the herb and shrub strata and *Tetragnatha laboriosa* and *Anyphaena rubra* in the herb stratum. It will be recalled that in the prevernal period Epitrix and Phalacrus were subdominants of the ground stratum. The other forms here mentioned, with the exception of Tetragnatha, were also in the ground stratum during the prevernal period. The same is true of *Xysticus elegans*, *Harpalus erythropus*, *Corimelaena pulicaria* and *Blissus leucopterus* which become subdominants during the vernal period.

The period between June first and September first is divided rather roughly into two parts, the first characterized by rising temperatures and increasing evaporating power of the air, and the second by a reversal of these changes. The former may be called the aestival period and the latter the serotinal. The population of the forest during this time is not nearly as great as during the preceding and following periods due to the absence of migratory forms. It is composed almost entirely of animals remaining in the forest throughout their life cycles.

A considerable number of species persist throughout the entire period. Of these the most prominent in the herb and shrub strata is *Epeira gibberosa*. Several other spiders including *Xysticus elegans*, now in the herb and shrub strata as well as below, *Pisaura undata* in the herb stratum, and *Acrosoma rugosa* in the shrub stratum, are found in sufficient numbers to entitle them to rank as subdominants. A few flies, such as *Sympycnus lineatus*, *Sapromyza fraterna* and *Minettia lupulina* are fairly abundant in the herb stratum but not as subdominants. Ants, *Camponotus herculeanus pennsylvanicus*, *Formica fusca*, *Lasius niger americanus*, and *Ponera coarctata pennsylvanica*, are sufficiently numerous to be considered as subdominants of the ground strata. The aestival period is also characterized by a large number of flies not taken at other times of the year. Among these are *Tipula mingwe*, *T. flavoumbrosa*, *Sapromyza notata*, *Psilopus tener* and *Dolichopus scapularis*.

During the serotinal period many additional forms appear, prominent among which are the lantern flies *Acanalonia conica*, *Ormenis pruinosa* and *O. septentrionalis* in the herb stratum. Here also appear *Linyphia phrygiana*, *Anyphaena rubra*, and *Dendryphantes aestivalis* which are about equally distributed in the herb and shrub strata, and a few other spiders. Other species present at this time are the flies *Sapromyza fraterna*, and *Psilopus scintillans* in the herb stratum, the beetles *Epurea rufa* in the leaf stratum and *Phytonomus nigrirostris* in the leaf and herb strata. The last named is an early migrant from the clover fields. None of those just mentioned are present in sufficient numbers to be considered sub-dominant but there is a sufficient differentiation between the make-up of the community during the earlier and later parts of the summer to warrant a distinction between the two phases of the summer society.

The meteorological characteristics of the autumnal period and their relations to the inward and downward moments of the migratory forms have been discussed at length in the section on the census of the animal population. The autumnal society is again dominated by the species migrating to the woodland for hibernation, and those animals which pass their entire life history in the forest are obscured by the migrants. The principal subdominants are the beetles *Phalacrus politus*, *Epitrix brevis*, *Notoxus monodon*, and *Telephanus velox*, and the leaf-hoppers *Empoasca viridescens* and *Erythroneura obliqua*. Phalacrus, Epitrix and the leaf-hoppers are found first in the shrub stratum, and thereafter in the herb and leaf strata. Notoxus and Telephanus are found first in the herb stratum and then in the leaves. A discussion, with especial reference to migration, of these species and others occupying somewhat less prominent places in the community, has already been given and the reader is referred to that section of the paper for further information in regard to the constitution and stratification of the autumnal society.

The animals spending the winter portion of their life cycles in the elm-maple association might be considered as making up an additional society, the hibernal. While none of these forms exhibit any great degree of activity, many become active on warm days, especially. The dominants of the winter period are the Hemiptera *Empoasca viridescens*, *Erythroneura obliqua*, *Blissus leucopterus*, *Lygus pratensis*, and *Corimelaena pulicaria*, the beetles *Phalacrus politus*, *Telephanus velox*, *Epitrix fuscula*, and *Notoxus monodon*, the fly *Leptocera evanescens* and the spiders *Xysticus elegans*, *Tetragnatha laboriosa* and *Anyphaena rubra*. All of these are found in the ground stratum, particularly under the shelter of the layer of fallen leaves, with the exception of Tetragnatha, which is found in the herb and shrub strata.

The animal communities of the elm-maple forest are characterized by great seasonal variation. As far as the lower strata, at least, are concerned, the changing composition of the seasonal societies obscures the vertical stratification. As mentioned previously, the same forms may appear successively in all three of the strata, and the same organism may be, at different seasons, a subdominant in different strata. This is especially true of the forms which migrate to and from the forest edge. A summary of the seasonal and stratal societies is given below:

Shrub Stratum	Herb Stratum	Ground Stratum
Prevernal Societies		
Tetragnatha laboriosa	*Tetragnatha laboriosa*	*Phalacrus politus*
		Epurea rufa
		Epitrix brevis
		Notoxus monodon
		Erythroneura obliqua
		Xysticus elegans
Vernal Societies		
Corythucha aesculi	*Epitrix brevis*	*Harpalus erythropus*
Diabrotica vittata	*Glyptina spuria*	*Corimelaena pulicaria*
	Diabrotica vittata	
Aestival Societies		
Epeira gibberosa	*Epeira gibberosa*	*Xysticus elegans*
Xysticus elegans	*Xysticus elegans*	*Camponotus herculeanus*
Acrosoma rugosa	*Pisaura undata*	*Formica fusca*
		Lasius niger
Serotinal Societies (In addition to above)		
	Acanalonia conica	
	Ormenis pruinosa	
	Ormenis septentrionalis	
Autumnal Societies		
Phalacrus politus →	*Phalacrus politus* →	*Phalacrus politus*
Epitrix brevis →	*Epitrix brevis* →	*Epitrix brevis*
Empoasca viridescens →	*Empoasca viridescens* →	*Empoasca viridescens*
Erythroneura obliqua →	*Erythroneura obliqua* →	*Erythroneura obliqua*
	Notoxus monodon →	*Notoxus monodon*
	Telephanus velox →	*Telephanus velox*
Hibernal Societies		
Tetragnatha laboriosa	*Tetragnatha laboriosa*	*Empoasca viridescens*
		Erythroneura obliqua
		Blissus leucopterus
		Lygus pratensis
		Corimelaena pulicaria
		Phalacrus politus
		Telephanus velox
		Epitrix fuscula
		Notoxus monodon
		Leptocera evanescens
		Xysticus elegans
		Anyphaena rubra

A further list of species taken in the forest, including those whose numbers were too small for mention among the dominants is given below. All forms are listed according to the stratum in which they were found.

List of Species Taken in University Woods. July 1, 1921 to July 1, 1922, with dates.

GROUND STRATUM

Diptera

Agromyza sp? larva (Agromyzidae) (Nov. 14).
Bibio albipennis Say, larva (Bibionidae) (Oct. 31; Nov. 21; Dec. 19).
Atrichopogon sp. undescribed (Chironomidae—Midge) (November 21).
Mycetophila punctata Meig. (Mycetophilidae—Fungus gnat) (November 7, 21; Dec. 19; Jan. 23).
Geosargus virdis Say, larva (Stratiomyidae) (Sept. 19; Oct. 3)
Microchrysa sp? larva (Stratiomyidae) (July 11).
Chionea sp? (Tipulidae—Wingless crane-fly) (Nov. 14).

Hemiptera

Catarhintha mendica Stål (Coreidae) (Oct. 3).
Corimelaena pulicaria Germ. (Cydnidae—Burrower-bug) (July 11; Aug. 22; Oct. 3, 17; Nov. 7, 14, 21, 28; Dec. 19; Jan. 2, 9, 16, 23, 30; Feb. 6; Mar. 27; April 3, 10, 17, 24; May 1, 8).
Aphanus umbrosus (Dist.) (Lygaeidae) (Sept. 12).
Blissus leucopterus (Say) (Lygaeidae—Chinch-bug) (Sept. 12; Oct. 3, 17, 31; Nov. 7, 14, 21; Dec. 5, 12, 19; Jan. 2, 9; Feb. 6; Mar. 27; April 24; May 8).
Nabis annulatus Reut. (Nabidae—Damsel-bug) (July 11, 18).
Euschistus euschistoides (Voll.) (Pentatomidae—Shield-bugs) (Oct. 17; March 27).
Menecles incertus (Say) (Pentatomidae) (July 4, 25; Aug. 1; May 22).
Corythucha ciliata (Say) (Tingidae—Lace-bug) (Oct. 3).
Erythroneura comes var. ziczac Walsh (Cicadellidae—Leaf-hoppers).
Erythroneura obliqua var. noevus Gill (Cicadellidae) (Oct. 31; Nov. 9; Jan. 2, 9, 23; Feb. 6, 20, 27).

Hymenoptera (Formicidae—Ants).

Aphaenogaster fulva Rog. (Aug. 8; Sept. 19; May 29; June 26; July 4).
Camponotus caryae Fitch var? (Aug. 22, 29; Sept. 12, 19; Oct. 3; June 5, 26; July 4).
Camponotus herculeanus pennsylvanicus DeGeer (July 11; Aug. 8; June 12; July 4).
Crematogaster lineolata Say (Sept. 26).
Formica fusca L. (July 4; Aug. 8, 29; June 12, 19, 25).
Lasius niger L. var. americanus Emery (July 4, 25; Aug. 29).
Lasius umbratus mixtus Nyl var. aphidicola Walsh (Nov. 28; June 19).
Myrmica punctiventris Rog. (July 11, 25; Aug. 8, 22, 29; Sept. 12, 19; Oct. 3; April 27; May 15; June 19, 26; July 4).
Myrmica scabrinodis schencki Emery var. emeryana Forel (July 18, 25; Aug. 8, 22, 29; Sept. 12, 26; Oct. 3; Nov. 21; Jan. 2; March 27; June 5; July 4).
Ponera coarctata pennsylvanica (Buckley) Emery (July 11; Aug. 8, 22; Jan. 9; May 8).
Prenolepis imparis Say (Sept. 12).

Coleoptera

Agonoderus pallipes Fab. (Carabidae—Ground Beetles) (Oct. 31).
Anisodactylus baltimorensis Say (Carabidae) (Oct. 31).
Anisodactylus carbonarius Say (Carabidae) (Sept. 19).

Anisodactylus interstitialis Say (Carabidae) (Aug. 1; Sept. 19; March 27; May 8).
Badister reflexus Lec. (Carabidae) (Feb. 6).
Blechrus nigrinus (Mann) (Carabidae) (Jan. 30).
Casnonia pennsylvanica L. (Carabidae) (Oct. 31; Nov. 14; Dec. 5, 26; Jan. 9; Feb. 6).
Clivina bipustulata Fab. (Carabidae) (Oct. 31; April 10).
Dicaelus furvus Dej. (Carabidae) (Aug. 8; May 29; June 12.)
Dicaelus purpuratus Bon. (Carabidae) (Aug. 8).
Evarthrus sigillatus (Say) (Carabidae) (July 4).
Harpalus erythropus Dej. (Carabidae) (July 25; Aug. 29).
Lebia atriventris Say (Carabidae) (Nov. 7).
Lebia grandis Hantz (Carabidae) (April 10).
Platynus basalis (Lec.)? (Carabidae) (Dec. 19).
Platynus 8-punctatus Fab. (Carabidae) (Oct. 17, 31; Jan. 23).
Tachys nubifer Csy.? (Carabidae) (Oct. 3).
Triliarthrus badiipennis (Hald.) (Carabidae) (Feb. 6).
Parandra brunnea Fab. (Cerambycidae—Long-horn beetle) (Dec. 5).
Cassida bivittata Say (Chrysomelidae—Leaf-beetle) (Oct. 31; Jan. 16).
Cerotoma trifurcata (Forst.) (Chrysomelidae) (Nov. 7; Jan. 30).
Chelymorpha argus Herbst (Chrysomelidae) (July 7; Jan. 9; March 27).
Lema trilineata Oliv. (Chrysomelidae) (Oct. 31; Jan. 9).
Metriona bicolor (Fab.) (Chrysomelidae) (Jan. 9, 23; Feb. 6, 13).
Oedionychis scalaris, Melsh. (Chrysomelidae) (March 6).
Oedionychis 6-maculata (Ill.) (Chrysomelidae) (Feb. 6, 27; March 27).
Phyllotreta sinuata Steph. (Chrysomelidae) (July 18; Oct. 3, 17, 31; Nov. 7, 28; Jan. 2, 9; Feb. 6).
Cincindela 6-guttata Fab. (Cincindelidae—Tiger Beetle) (June 12).
Hyperaspis undulata Say (Coccinellidae—Lady-bird) (Nov. 7).
Silvanus surinamensis L.? (Cucujidae—Grain beetle) (Nov. 14).
Conotrachelus erinaceus Lec (Curculionidae—Weevils) (Feb. 6; March 27).
Conotrachelus seniculus Lec. (Curculionidae) (Oct. 13).
Lixus terminalis Lec. (Curculionidae) (March 27).
Copelatus glyphicus Say (Dytiscidae—Predacious diving beetle) (July 18).
Agriotes insanus Cand. (Elateridae—Click-beetles) (March 27).
Asaphes memnonius Herbst (Elateridae) (July 25).
Drasterius sp? (Elateridae) (Oct. 31; Nov. 7; Jan. 9, 16).
Glyphonyx testaceus Melsh. (Elateridae) (Sept. 19; Jan. 9, 16; Feb. 27).
Melanotus communis Gyll. (Elateridae) (June 12).
Hister americanus Payk. (Histeridae) (May 1).
Hister vernus (Say) (Histeridae) (Oct. 31).
Lucidota atra (Fab.) (Lampyridae—Firefly) (June 19).
Glischrochilus fasciatus (Oliv.) (Monotomidae) (Feb. 6).
Carpophilus antiquus (Melsh.) (Nitidualidae) (Feb. 6).
Epurea rufa Rest. (Nitidulidae) (July 11; Aug. 22; Sept. 19; Oct. 3, 31; Nov. 14, 21; Jan. 2; Feb. 6; March 27; April 10).
Stelidota geminata (Say) Nitidulidae) (May 1, 8).
Stelidota 8-maculata Say (Nitidulidae).(July 4).
Pilopius piceus (Lec.) (Pselaphidae) (July 4, 11; Aug. 29; Sept. 12; Feb. 6; April 3).
Serica sericea (Ill.) (Scarabaeidae—Leaf-chafers) (March 27; April 17).
Xyloryctes satyrus (Fab.) (Scarabaeidae) (June 5).
Colon sp? (Silphidae—Carrion-beetle) (July 4).
Lathrobium collare Er.? (Staphylinidae—Rove-beetles) (April 3).

Lathrobium longiusculum Grav. (Staphylinidae) (Oct. 31; Dec. 5; April 10; May 1, 8).
Lathrobium simplex Lec.? (Staphylinidae) (July 4, 11).
Mycetoporus humidus (Say)? (Staphylinidae) (Feb. 6).
Olophrum obtectum Er. (Staphylinidae) (May 1; June 12).
Staphylinus viridianus Horn (Staphylinidae) (July 4).
Stilicus dentatus Say (Staphylinidae) (Nov. 7).
Tachyporus jocosus Say (Staphylinidae) (July 25; Aug. 1; March 27; June 19).
Tachinus pallipes Grav. (Staphylinidae).

Spiders

Cicurina arcuata Keys. (Agelenidae—Funnel-web spiders) (Dec. 26; Feb. 6, 27; March 27; June 19).
Hahnia agilis Keys. (Agelenidae—funnel-web spiders) (July 4, 25).
Hahnia cinerea Emer. (Aug. 22; Sept. 5; Oct. 17; Jan. 23; Feb. 6; April 10).
Castaneira bivittata, Keys. (Clubionidae) (Aug. 22; Sept. 19; Oct. 3; Feb. 13; April 3).
Phrurolithus alarius Hantz (Clubionidae) (Oct. 31; March 27; April 10; June 19).
Prosthesima rufula Banks (Drassidae) (Feb. 27; May 8).
Erigone sp? (Linyphiidae) (Nov. 7, 21; March 27).
Lephthyphantes sp? (Linyphiidae) (Nov. 14; April 24).
Microneta cornupalpis Camb. (Linyphiidae) (Dec. 5).
Lycosa lepida Keys. (Lycosidae—Wolf spiders) (Jan. 23; March 13).
Lycosa rubicunda Keys. (Lycosidae) (Feb. 27; March 27; April 10; May 1).
Pardosa sp? (Lycosidae) (July 11, 18; Aug. 1, 29; Oct. 3; Nov. 7; March 27).
Pirata sp? (Lycosidae) (Oct. 31; Jan. 9; Feb. 6).
Ceratinella sp? (Theridiidae—Comb-footed spider) (May 1).
Oxyptila conspurcata Thor. (Thomisidae—Crab-spider) (Oct. 31; Nov. 7; April 3, 24).

Myriapoda

Chilopoda (Centipedes).
Arenophilus bipuncticeps (Wood) (Oct. 31).
Bothropolys multidentatus (New.) (Aug. 8; Nov. 21; Jan. 16).
Linotaenia chionophila (Wood) (July 4, 11; Aug. 8; Nov. 21; Dec. 19; Feb. 6, 20).
Linotaenia fulva (Gager) (Aug. 8; Sept. 19; Oct. 17).
Notobius iowensis (Mein.) (July 4; Aug. 1, 8, 22; Sept. 19; Dec. 12; Feb. 6).
Otocryptops sexspinosus (Say). (July 4, 18; Aug. 22; Sept. 12, 19; Oct. 3; Dec. 5).
Poabius Vilabiatus (Wood) (July 11; Oct. 31; Nov. 28).
Diplopoda (Millipedes).
Cleidogona caesionnulata (Wood) (July 4, 11; Nov. 7).
Fontaria virginiensis (Drury) (Aug. 22).
Scytonotus granulatus (Say) (Aug. 22; Oct. 31; Nov. 7, 21, 28; Dec. 12).
Spirobolus marginatus (Say) (July 4, 18, 15; Aug. 29; Sept. 12, 19).
Striarea sp? (Oct. 31).

Mollusca

Pulmonate Snails.
Carychium exiguum (Say) (Auriculidae) (Oct. 31).
Agriolimax agrestis (Linn.) (Limacidae—Slug) (Nov. 7).
Phylomycus caroliniensis (Bosc.) (Phylomycidae—Slug) (Aug. 29; Oct. 31; Nov. 7, 14, 28).
Bifidaria pentodon (Say) (Pupidae) (July 11).
Succinea avara Say (Succineidae) (July 11; Oct. 3).
Zonitoides arborea (Say) (Zonitidae) (July 7; Aug. 8).
Zonitoides minuscula (Binn.) (Zonitidae) (July 4, 11; Jan. 2).

GROUND AND HERB STRATA

Diptera

Leptocera evanescens Tuck. (Borboridae) (Oct. 3; Nov. 7, 14, 28; Dec. 5, 12, 19; Feb. 6, 20).
Apiochaeta sublutea Mall. (Phoridae—Hump-backed fly) (Nov. 7; June 26).
Trichocera brumalis Fitch (Tipulidae—Crane-fly) (Oct. 31; Nov. 7; 21).

Hemiptera

Myodocha serripes Oliv. (Lygaeidae) (Oct. 31; Nov. 14, 28; Feb. 6, 27; March 27; April 3, 10; June 12).
Dicyphus gracilentus Parsh. (Miridae) (Type locality) (July 4; Aug. 8; Sept. 5, 19, 26; May 1; June 26).
Nabis ferus (L.) (Nabidae—Damsel bugs) (Oct. 17; Nov. 7, 28; Dec. 5, 12; Jan. 30).
Nabis roseipennis Reut. (Nabidae—Damsel bugs) (Oct. 31; Nov. 7, 14; Jan. 16; Feb. 6; March 13).
Podisus maculiventris (Say) (Pentatomidae—Soldier bug) (Oct. 17; Nov. 14; Jan. 9; Feb. 6, March 6).
Piesma cinera (Say) (Tingidae—Lace-bug) (Sept. 26; Oct. 3; Nov. 14).
Erythroneura obliqua var. parma McAtee (Cicadellidae) (Oct. 3; Dec. 5, 12; Jan. 2, 9, 23; Feb. 6).
Erythroneura vulnerata Fitch (Cicadellidae).
Scaphoideus scalaris V.D. (Cicadellidae)
Thamnotettix longulus G. & B. (Cicadellidae) (Sept. 19; Nov. 21).

Coleoptera

Notoxus monodon Fab. (Anthicidae) (Aug. 29; Sept. 12, 19, 26; Oct. 3, 10, 17, 31; Nov. 7, 21, 28; Dec. 5, 12, 19; Jan. 2; Feb. 6, 20, 27; March 27; April 3, 10, 17; May 1).
Tomarus pulchellus Lec. (Cryptophagidae) (Sept. 19; Nov. 7; Jan. 9; Feb. 20; April 3, 10).
Telephanus velox Hald. (Cucujidae) (Oct 3, 31; Nov. 7, 14, 21, 28; Dec. 19; Jan. 9, 30; Feb. 6, 20).
Phytonomus nigrirostris Fab. (Curculionidae—Lesser clover-leaf weevil) (July 4, 11, 18; Aug. 22, 29; Sept. 12, 19, 26; Oct. 3, 31; Dec. 12, 19; Jan. 9, 23; Feb. 20, April 10, 17).
Hypera punctata Fab. (Curculionidae) (Aug. 29; Sept. 12; Oct. 31; Feb. 6).
Phontinus scintillans Say (Lampyridae—Fire-fly (June 26; July 4).

Spiders

Agelena naevia Walck. (Agelenidae—Grass spider) (Sept. 5, 12; Oct. 3; March 13; June 19).
Phidippus multiformis Emer. (Attidae—Jumping spider) (July 11, 25; May 1).

Mollusca

Circinaria concava (Say) (Circinariidae) (July 25; Aug 8; Sept. 12, 19; Oct. 3, 17; Nov. 28; Jan. 9).
Vitrea indentata (Say) (Zonitidae) (July 11, 25; Aug. 29; Sept. 12, 19; Oct. 3, 17, 31; Nov. 7, 14, 28; Dec. 5; Jan. 23).

GROUND, HERB AND SHRUB STRATA

Diptera

Orthellia caesarion Meig. (Muscidae—Blue-bottle fly) (Nov. 7, 28; Jan. 6, 23).
Sciara sp? (Mycetophilidae—Fungus gnat) (Aug. 29; Sept. 19, 26; Oct. 10, 31; Nov. 7; April 3).

Hemiptera

Acanthocephala terminals (Dall) (Coreidae) (Aug. 8, 22, 29; Sept. 12, 26; Oct. 3, 10, 17, 24, 31; Feb. 6).

Lygus pratensis (L.) (Miridae—Tarnished plant-bug) (Oct. 3, 10, 31; Nov. 7, 28; Dec. 5, 12; Jan. 2, 16; Feb. 6, 13; March 13, 27).

Nabis sordidus Reut. (Nabidae—Damsel bug) (Aug. 8, 29; Sept. 5; Nov. 14).

Euschistus variolarius (P. B.) (Pentatomidae—Soldier bug) (Aug. 29; Nov. 7, 21, 28; Dec. 5, 12; Feb. 6; March 27; April 3, 10, 17, 24).

Hymenarcys aequalis (Say) (Pentatomidae) (Aug. 29; Oct. 31; Nov. 7; Dec. 12; Feb. 6; May 8).

Zelus exsanguis (Stål) (Reduviidae—Assassin-bug) (July 18, 25; Aug. 29; Sept. 26; Oct. 3, 10, 17, 31).

Corythucha aesculi O. & D. (Tingidae—Lace-bug) (July 4, 18; Sept. 12, 19; Oct. 17; Jan. 9; Feb. 13; April 3, 10, 24; May 1, 8; June 5, 12, 19).

Gargaplia tiliae (Walsh) (Tingidae) (Sept. 19, 26; Oct. 31; Nov. 7; Dec. 19).

Empoasca viridescens Walsh (Cicadellidae—Leaf-hoppers) (Sept. 19, 26; Oct. 3, 17, 31; Nov. 7, 14, 21, 28; Dec. 5, 19; Jan. 30; Feb. 6; Mar. 6).

Erythroneura comes var. basilaris (Say) (Cicadellidae).

Erythroneura comes var. maculata (Gill.) (Cicadellidae).

Erythroneura comes var. vitis Harr. (Cicadellidae) (Oct. 17; Nov. 28).

Erythroneura obliqua var. scutellaris (Gill.) (Cicadellidae).

Coleoptera

Lebia ornata Say (Carabidae—Ground beetles) (Dec. 9; Oct. 31; March 27; June 12).

Lebia scapularis Dej. (Carabidae) (Sept. 19, 26; Oct. 3; Jan. 3).

Chaetocnema confinis Crotch. (Chrysomelidae—Leaf-beetles) (Sept. 19, 26; Oct. 11; Nov. 14; May 8).

Diabrotica ittata Fab. (Chrysomelidae) (Aug. 29; Sept. 12, 26; Oct. 3, 10, 17, 31; April 10; May 1, 8; June 5).

Epitrix brevis Schw. (Chrysomelidae) (Sept. 19, 26; Oct. 3, 17; Jan. 6, 16, 23, 31; April 10; May 8; June 12, 19, 26).

Epitrix fuscula Crotch. (Chrysomelidae) (Sept. 19; Oct. 3, 31; Nov. 7, 14, 21; Dec. 12, 19; Jan. 9; Feb. 27; May 8).

Glyptina brunnea Horn (Chrysomelidae) (Sept. 26; Oct. 17; Feb. 6; June 5, 19).

Glyptina spuria Lec. (Chrysomelidae—Leaf-beetles) (Sept. 12, 26; Oct. 31; Nov. 7, 21; Dec. 5, 12; Jan. 9; Feb. 27; March 6, 13, 27; April 3, 10, 17; May 1, 8; June 12).

Xanthonia villosula (Melsh.) (Chrysomelidae) (July 3, June 19, 26).

Lathridiidae unidentified (Sept. 12; Oct. 3; Nov. 7, 14; Dec. 12; Feb. 6; March 27; April 3; May 1, 8; June 26).

Phalacrus politus Melsh. (Phalacridae) (Sept. 26; Oct. 3, 10, 17, 31; Nov. 7, 14, 21, 28; Dec. 5, 12, 19; Jan. 2, 9, 16, 23; Feb. 6; March 27; April 10, 17; May 1, 8; June 12).

Spiders

Phidippus tripunctatus Emer. (Attidae—Jumping spider) (Sept. 26; Oct. 10, 17, 31; April 10).

Wala palmarum Hentz (Oct. 3, 31; Feb. 6; April 10; May 1).

Anyphaena rubra Emer. (Clubionidae) (July 19; Aug. 8, 29; Sept. 19, 26; Oct. 3, 10, 17, 31; Nov. 14, 21; Jan. 2, 16, 23, 30; Feb. 6, 20; March 6, 13, 27; April 10; May 1, 22).

Clubiona crassipalpus Keys. (Clubionidae) (May 15).

Dictyna muraria Emer. (Dictynidae) (Aug. 29; Sept. 5, 12, 19, 26; Oct. 3, 10, 17, 31; Nov. 21; Dec. 19; Jan. 2; Feb. 6; April 10; June 5).

Dictyna volupis Keys. (Dictynidae) (Feb. 20; March 6; May 15, 22, 29).

Sergiolus variegatus Hentz (Drassidae) (Oct. 10; Feb. 13, 27).

Epeira gibberosa Hentz (Epeiridae—Orb-weavers) (July 11, 18, 25; Aug. 8, 22, 29; Sept. 5, 12, 19; Nov. 7; Feb. 6; April 3; May 22; June 5, 12, 19, 26).

Tetragnatha laboriosa Hentz (Epeiridae) (July 11; Oct. 24; Nov. 7, 21, 28; Dec. 5, 19; Jan. 9; March 6, 13, 27; April 3, 24; May 1, 22).

Linyphia phrygiana Koch (Linyphiidae) (July 11, 18; Aug. 8, 29; Sept. 12, 19, 26; Oct. 10, 31; Nov. 7, 21, 28; Dec. 12; Jan. 9; Mar. 6; June 19).

Dolomedes idoneus Mont. (Pisauridae—Nursery-web weavers) (July 4, 11, 18, 25; Aug. 8, 29; Sept. 12, 19, 26; Oct. 17; Dec. 19; Feb. 6; March 27; April 10; June 12, 19).

Pisaura undata Hentz (Pisauridae) (July 11, 18; Aug. 1, 8, 22, 29; Sept. 12, 19, 26; Oct. 3, 17, 31; Dec. 19; Feb. 6; April 10; May 1; June 12, 19).

Philodromus ornatus Banks (Thomisidae—Crab-spiders) (Aug. 8, 29; Sept. 19; Oct. 3; June 26).

Xysticus elegans Keys (Thomisidae) (July 11, Aug. 1, 8, 15, 22, 29; Sept. 5, 12, 26; Oct. 10, 24; Nov. 7, 14, 21, 28; Dec. 5, 12, 19; Jan. 2, 9; Feb. 6, 20; March 27; April 10, 24; May 1, 15, 29; June 19, 26).

Vloborus americanus Walck. (Vloboridae) (Sept. 12, 19; Oct. 17).

Mollusca

Polygyra thryroides (Say) (Helicidae) (July 11, 18, 25; Aug. 8, 22, 29; Sept. 12, 19, 26).

HERB STRATUM

Diptera

Coenosia lata Walk. (Anthomyidae—Root-maggot flies) (May 1).
Macorcoenosia triseta Stein. (Anthomyidae) (May 8).
Phorbia fusciceps Zett. (Anthomyidae) (Nov. 28).
Borborus equinus Fall. (Borboridae) (June 12).
Camptocladius byosinus Schrank. (Chironomidae—Midges) (June 12).
Chironomus cristatus Fabr. (Chironomidae) (July 18).
Chironomus riparius Meig. (Chironomidae) (Sept. 26).
Chironomus sp? (undescribed) (Chironomidae) (July 3; Sept. 26).
Dexia vertebrata Say (Dexiidae) (July 18).
Dolichopus funditor Loew. (Dolichopodidae—Long-legged flies) (July 4).
Dolichopus longipennis Loew (Dolichopodidae) (June 12).
Dolichopus scapularis Loew (Dolichopodidae) (June 19, 26).
Chimomyza amoena Loew (Drosophilidae—Fruit flies) (June 12).
Drosophila melanica Sturt. (Drosophilidae) (Feb. 13).
Leucophenga varia Walk. (Drosophilidae) (Oct. 3).
Scaptomyza graminum Fall. (Drosophilidae) (Nov. 28; March 27).
Helomyza longipennis Loew (Helomyzidae) (June 12).
Heteromeringia sp? (Heteroneuridae) (June 26).
Chrysopilus modestus Loew (Leptidae—Snipe-fly) (June 26).
Boletina obscura Joh. (Mycetophilidae—Fungus gnat) (March 27).
Cordylura volucris Joh. (Mycetophilidae) (Nov. 21).
Exechia sp? (Mycetophilidae) (Nov. 21).
Mycetophila extincta Loew (Mycetophilidae) (June 12).
Neosciara sp? (Mycetophilidae) (June 5).
Batanobia pusilla Meig.? (Oscinidae) (Oct. 3).
Chloropisca glabra Meig. (Oscinidae) (Oct. 3; June 5).
Hippelastes flavipes Loew (Oscinidae) (Oct. 17; June 26).
Platypeza velutina Loew (Platypezidae—Flat-footed fly) (Oct. 3).
Sapromyza fraterna Loew (Sapromyzidae) (July 4, 18, 25; Aug. 8).
Sapromyza philadelphica Macq. (Sapromyzidae) (June 26).
Scatophaga furcata Say (Scatophagidae—Dung-flies) (April 24).
Scatophaga stercoraria L. (Scatophagidae) (Aug. 22).
Trypetoptera canadensis Macq. (Sciomyzidae) (June 12).
Sepsis violacea Meig. (Sepsidae) (Nov. 28).

Microphthalmia disjuncta Wied. (Tachinidae—Tachina-flies) (Aug. 22).
Sciasma nebulosa Coq. (Tachinidae) (Oct. 17).
Cladura flavoferruginea O.-S. (Tipulidae—Crane-flies) (Oct. 3, 17).
Dicranoptycha sobrina O.-S. (Tipulidae) (Aug. 22; June 19, 26).
Helobia hybrida Meig. (Tipulidae) (Oct. 17; Nov. 28).
Rhipidia domestica O.-S. (Tipulidae) (Oct. 24).
Tipula unimaculata (Loew) (Tipulidae) (Aug. 8).

Hemiptera
Triphleps insidiosus Say (Anthocoridae) (Oct. 3).
Geocoris uliginosus (Say) (Lygaeidae) (Sept. 26).
Macrolophus separatus (Uhl.) (Miridae) (July 11).
Acrosternum hilaris (Say) (Pentatomidae—Shield bug) (July 18).
Emesa brevipennis Say (Reduviidae—Assassin bug) (Aug. 8; Sept. 19).
Galgupha nitiduloides Wolff (Cydnidae—Burrower-bug) (July 11).
Balclutha punctata Thunb. (Cicadellidae—Leaf-hoppers) (Sept. 26).
Dikraneura carneola (Stål) (Cicadellidae). (Oct. 3).
Empoasca obtusa Walsh (Cicadellidae).
Stenocranus dorsalis (Thunb.) (Cicadellidae).
Acanalonia conica Say (Fulgoridae) (July 4, 11, 18).
Ormenis pruinosa Say (Fulgoridae) (July 11, 18; Aug. 29; Nov. 28).
Stobaera concinna Stål (Fulgoridae) (Dec. 19).

Hymenoptera
Chelonus sp? (Braconidae) (Sept. 26).
Cyanopterus sp? (Braconidae) (Aug. 15; Sept. 5).
Orgilus sp? (Braconidae) (Sept. 12).
Rhogas terminalis Cress. (Braconidae) (July 18).
Callimome sp? (Chalcidae) (Oct. 3).
Dibrachys boucheanus Ratz. (Chalcidae) (Oct. 17).
Tetrastichus sp? (Chalcidae) (Oct. 3).
Aenoplegimorpha sp? (Ichneumonidae) (Sept. 26).
Amblyteles mucronatus (Prov.) (Ichneumonidae) (Sept. 12).
Amblyteles w-album (Cress.) (Ichneumonidae) (Aug. 15).
Hoplismenus morulus (Say) (Ichneumonidae) (July 11).
Spilocryptus propodeum Cush. (Ichneumonidae) (Sept. 26).

Coleoptera
Calathus opaculus Lec. (Carabidae—Ground beetle) (Sept. 5).
Liopus fascicularis Harr. (Cerambycidae—Long-horn beetle) (Aug. 8).
Diabrotica 12-punctata (Fab.) (Chrysomelidae—Leaf-beetles) (Nov. 28).
Longitarsus melanurus Melsh (Chrysomelidae) (Sept. 26; Oct. 3; Nov. 28; April 10).
Oedionychis gibbitarsa Say (Chrysomelidae) (Oct. 10).
Rhabdopterus picipes (Oliv.) (Chrysomelidae) (July 4).
Chilocorus bivulnerus Muls. (Coccinellidae—Lady-beetles) (Oct. 3).
Megilla maculata DeGeer (Coccinellidae) (Nov. 21; June 19).
Acalles carinatus Lec. (Curculionidae—Weevils) (March 6).
Conotrachelus tuberosus Lec. (Curculionidae) (July 4).
Idiostethus subcalvus (Lec.) (Curculionidae) (May 8).
Idiostethus tubulatus (Say) (Curculionidae) (May 8).
Orchestes mixtus Blatch.? (Curculionidae) (June 26).
Byturus unicolor Say (Dermestidae) (June 19).
Limonius griseus Beauv.? (Elateridae—Click-beetles) (July 4).

Ludius attenuatus Say (Elateridae (July 4, 18).
Photinus pyralis L. (Lampyridae—Fire-flies) (July 4; June 19).
Pyractomena angulata (Say) (Lampyridae) (July 11; June 19).
Plateros canaliculus Say (Lycidae) (June 26; July 8).
Mordella triloba (Say) (Mordellidae) (July 4).
Brachypterus urticae Fab. (Nitidulidae) (Sept. 5, 12).
Brachytarsus sticticus Boh. (Platystomidae) (May 8).

Spiders
Maevia vittata Hentz (Attidae—Jumping spiders) (July 11; Aug. 29; May 29).
Plexippus puerperus Peck. (Attidae) (Sept. 19)
Anyphaena calcarata Emer. (Clubionidae) (May 8; June 26).
Argiope aurantia Lucas (Epeiridae—Orange garden spider) (July 1; Sept. 19).
Mimetus interfector Hentz (Mimetidae) (Sept. 19).
Theridion frondeum Hentz (Theridiidae) (Aug. 8).
Musumena asperata Hentz (Thomisidae—Crab-spiders) (Aug. 29; Sept. 12; June 19).
Misumena oblonga Keys. (Thomsidae) (June 12, 19).

Mollusca
Sphyradium edentulum (Drap.) (Endodontidae) (Oct. 17).
Vertigo milium (Gould) (Pupidae) (Oct. 3, 17).

HERB AND SHRUB STRATA

Diptera
Chironomus decorus Joh. (Chironomidae—Midge) (June 12, 19).
Pelastoneurus vagans Loew (Dolichopodidae—Long-footed flies) (Oct. 3; June 26).
Psilopus patibulatus Say (Dolichopodidae) (July 18; Aug. 22).
Psilopus scintillans Loew (Dolichopodidae) (July 25; Aug. 8, 22; Sept. 5).
Psilopus tener (Loew) (Dolichopodidae) (June 19, 26).
Sympychus lineatus Loew (Dolichopodidae) (Sept. 26; Oct. 3; May 1, 8; June 5, 12).
Batanobia coxendix (Fitch) (Oscinidae). (April 3).
Minettia lupulina Fab. (Sapromyzidae) (Aug. 8; June 19, 26).
Dicranoptycha winnemana Alex. (Tipulidae—Crane-flies) (July 18; June 15).
Tipula mingwe Alex. (Tipulidae) (June 5).
Tipula flavoumbrosa Alex. (Tipulidae) (June 5, 19).

Hemiptera
Paraxenetus guttulatus (Uhl.) (Miridae) (July 4, 11).
Jalysus spinosus (Say) (Neididae—Stilt-bug) (July 25; Aug. 8; Sept. 26; Jan. 9).
Deltocephalus inimicus Say (Cicadellidae—Leaf-hoppers) (Aug. 29; Sept. 12, 26).
Empoasca mali (LeB.) (Cicadellidae).
Empoasca rosae (L.) (Cicadellidae).
Gypona 8-lineata (Say) (Cicadellidae) (Aug. 25; June 16).
Scaphoideus auronitens Prov. (Cicadellidae) (July 4; Aug. 8).
Acanalonia bivittata Say (Fulgoridae) (July 4).
Ormenis septentrionalis Spin. (Fulgoridae) (July 4, 11, 18; Aug. 29; Nov. 28).

Hymenoptera
Microbracon sp? (Braconidae) (July 4; Sept. 26).
Ephialtes aequalis (Prov.) (Ichneumonidae) (Sept. 5, 12, 26).

Coleoptera
Podabrus rugulosus Lec. (Cantharidae) (June 5).

Chalepus nervosa Panz. (Chrysomelidae—Leaf-beetle) (July 4; Aug. 22, 29; Sept. 5, 12, 26).
Psyllobora 20-maculata (Say) (Coccinellidae—Lady-beetle) (May 8).
Apion sp? (Curculionidae—Weevils) (Sept. 26; Oct. 3, 10, 17; May 8; June 12, 26).
Gelus oculatus Say (Curculionidae) (Aug. 29; Oct. 17).
Mordellistena tosta Lec. (Mordellidae) (June 19, 26).

Spiders

Dendryphantes aestivalis Emer. (Attidae—Jumping Spiders) (Aug. 8, 22, 29; Sept. 5, 12, 19, 26; Oct. 10, 17; April 3; May 1, 22, 29).
Habrocestum pulex Hents (Attidae) (Aug. 29; Sept. 12, 19).
Zygoballus bettini Peck. (Attidae). (July 15; Aug. 22, 29; Sept. 5, 26; June 5).
Acrosoma rugosa Hentz (Epeiridae—Orb-weavers) (July 11; Aug. 8, 22, 29; Sept. 19; Oct. 10; June 19).
Acrosoma spinea Hentz (Epeiridae) (Aug. 15: June 19).
Epeira hortorum Hentz (Epeiridae). Aug. 29; Sept. 12, 19; Oct. 10, 17; Nov. 28; Feb. 20; May 1; June 12, 19).
Linyphia communis Hentz (Linyphiidae) (Sept. 12, 19; Oct. 17).
Tmarus caudatus Hentz (Thomisidae—Crab-spiders) (Oct. 31; March 27).
Hyptiotes cavatus Hentz (Uloboridae) (Sept. 12; Oct. 17; Jan. 9; Feb. 6; May 29; June 12).

LEAF AND SHRUB STRATA

Sceptonia nigra Meig. (Mycetophilidae—Fungus gnat). (Oct. 24, 31; Jan. 2).
Apanteles sp? (Braconidae—Parasitic Hymenopteron). (July 18; Sept. 26).
Caenocara bicolor Germ (Anobiidae—Beetle). (Oct. 17; Nov. 14).
Lina lapponica (L.) (Chrysomelidae—Leaf-beetle). (July 18; Oct. 17; Nov. 21; Dec. 5, 12; Jan. 16; June 26).

SHRUB STRATUM

Diptera

Xenocoenosia calopiga Loew (Anthomyidae—Root-maggot fly) (June 26).
Bibio fraternus Loew (Bibionidae) (May 8).
Forcipomyia specularis Coq. (Chironomidae—Midge) (Sept. 26).
Drosophila quinaria Loew (Drosophilidae—Fruit fly) (Oct. 31).
Allodia falcata Joh. (Mycetophilidae—Fungus gnats) (Nov. 28).
Sciara sciophila Loew (Mycetophilidae). (June 5).
Cetema procera Loew (Oscinidae). (June 12).
Calipe gracilipes (Loew) (Sapromyzidae) (June 26).
Alophora aeneoventris Will. (Tachinidae) (May 1).

Hemiptera

Euschistus tristigmus (Say) (Pentatomidae—Shield-bug) (Aug. 29).
Sinea spinipes (H.-S.) (Reduviidae—Assassin-bug) (Aug. 29).
Erythroneura tricincta var. catycula McAtee (Cicadellidae—Leaf-hoppers) (Sept. 26).
Graphocephala versuta (Say) (Cicadellidae) (Nov. 21).
Gypona pectoralis Spanbg. (Cicadellidae).
Phlepsius irroratus Say (Cicadellidae) (Aug. 29).
Scaphoideus immixtus Say (Cicadellidae).

Hymenoptera

Rhogas intermedius Cress (Braconidae) (Sept. 26).
Campoplex sp? (Ichneumonidae) (Oct. 3).
Epiurus sp? (Ichneumonidae) (Oct. 31).

Plectiscus sp? (Ichneumonidae) (Oct. 31).
Thymarus sp? (Ichneumonidae) (Sept. 19).

Coleoptera
Telephorus lineola Fab.? (Cantharidae) (July 4).
Scymnus fraternus Lec. (Coccinellidae—Lady-beetle) (May 1, 8).
Melanotus ignobilis (Melsh.) (Elateridae—Click-beetle) (June 26).
Ptilodactyla serricollis (Say) (Helodidae) (June 12).
Mordellistena trifasciata (Say) (Mordellidae) (June 19).
Pentaria trifasciata (Melsh.) (Mordellidae) (June 26).
Osmoderma scabra (Beav.) (Scarabeidae—Routh flower-beetle) (July 4).

Spiders
Wala mitrata Hentz (Attidae—Jumping Spiders) (Oct. 10, 31).
Epeira cornigera Hentz (Epeiridae—Orb-weavers) (Oct. 31).
Epeira domiciliorum Hentz (Epeiridae) (Aug. 22, 29; Sept. 12).
Epeira insularis Hentz (Epeiridae) (Sept. 12, 19; Oct. 10).
Epeira prompta Hentz (Epeiridae) (Oct. 10; Dec. 5; Jan. 30; March 13; June 19).
Epeira stricta Hentz (Epeiridae) (Sept. 26; Oct. 10, 17).
Bathyphantes micaria Emer. (Linyphiidae) (June 19).
Euriopis funebris Hentz (Theridiidae) (Oct. 10).
Steatoda borealis Hentz (Theridiidae) (Sept. 19).
Coriarachne versicolor Keys. (Thomisidae—Crab-spiders) (Sept. 19).
Ebo latithorax Keys. (Thomisidae) (Oct. 10).

It appears then that the animal sub-dominants in the elm-maple association are, during a considerable portion of the year, largely species which have migrated from or are in the course of migration to or from the forest edge or the adjacent meadows. The ecotone between forest and meadow has its own fauna as well as its own flora, of which the former is concerned not only with the forest border proper, but with a more extensive zone extending outward to the meadow and inward to the depths of the forest. In addition to the animals observed in this study, many larger forms have been observed to frequent and breed in the forest margin region. Shelford (1913) lists seven species of mammals and about thirty species of birds which breed in the forest or at the forest edge and feed in the open meadows or prairies. (See also Wood, (1910).)

The community of the forest edge is relatively permanent and relatively extensive, as permanent and as extensive as the savanna. But little forest or meadow within the savanna is far enough from the other chief association (or associes) of the region not to be dominated at some season of the year by those forms for the completion of whose life history both types of habitat are required. From the animal standpoint we then have the same subdominants occurring throughout the savanna, and some of the same societies occurring in both the grassland and forest associations, at different seasons of the year. From the animal standpoint the savanna is a unit community, dominated by forms, the adjustment of whose life cycles to the climatic rhythm of the region is similar, identical or equivalent.

If the prairie region of the savanna is to be considered as an associes whose developmental processes are directed toward the deciduous forest climax, the forest communities bordering the prairies must also receive the designation of associes rather than associations. Whatever process of development affects one of these communities affects the other and the replacement of grassland by woodland in the forest border region would shift the range of migration of the species comprising the distinctive fauna of the savanna in the direction of the new dividing line between forest and prairie. A certain portion of woodland would thus lose its migrant population, with the consequent change in societies and subdominants, if not in dominants and become a part of the deciduous forest formation in its restricted sense.

The chief criterion, according to Clements, of the climax formation (plant) is the identity of vegetation-form of the dominants. Also "one or more of the dominant species must range throughout the formation as a dominant to a larger or smaller degree . . . The majority of the dominant genera extend throughout the formation . . . Most of the subdominants belong to the same genera. . . ."

No criterion closely comparable to vegetation-form can be applied to animal dominants. However, vegetation-form is a fundamental response to climate. The fundamental response to climate on the part of an animal is the character of its life cycle, the adjustment of the annual rhythm of the animal to the climatic rhythm of the habitat. We have seen that the adjustment of the life cycles of savanna animals to the climatic rhythm is definite and similar in so far as sub-dominants of the same seasonal societies are concerned. It is equivalent for subdominants belonging to different societies. The dominant animals, under primitive conditions, are now almost entirely absent. Their life cycles, however, were similarly adjusted to the climatic rhythm. Most of the subdominants are very widely distributed over the savanna region, and the dominants ranged similarly. It would seem, according to the above, that the animal community of the temperate savanna might be considered as an animal formation probably in the subclimax stage with the woodland and meadow associations as alternes. However, before a final terminology is adopted, it is necessary that more work be done in the correlation of the animal and plant communities of the region. Formations should not be delimited on the basis of vegetation-form alone, but the life histories of the dominant animals should be considered as well. The final solution of the problem requires the cooperative effort of plant and animal ecologists.

EXPERIMENTAL STUDIES

Reactions of Animals in Gradients

In spite of their many points of unsuitability for experimental work, spiders were chosen on account of their definite vertical distribution for experiments involving gradients in environmental conditions. Practically all of the web-building species show narrowly limited stratal relations. As examples the height above the ground of the web of the following species taken in the area considered may be cited:

Species	Average Height m	Maximum Height m	Minimum Height m
Acrosoma rugosa	1.25	2.25	0.3
Acrosoma spinea	0.7		
Linyphia phrygiana	1.5	3.0	0.3
Anyphaena	1.25	3.0	0.3
Uloborus americanus	0.7	2.0	0.3
Epeira gibberosa	0.7	2.0	0.3
Hyptiotes cavatus	1.0	1.5	0.3

These averages are based on a large number of collections, with the exception of *Acrosoma spinea*, which is included here only for comparison with *A. rugosa*. According to Shelford (1913) this species is usually found still lower.

Of the species mentioned above only a part were suitable, on account of abundance, size, etc., for prolonged experimentation. Faults for this purpose of some of the species sufficiently abundant were small size, pugnacity, sluggishness, tendency to build a maze of web in the experimental cage or to make a silken retreat in a corner of the cage no matter what the air conditions might be. One familiar with the behavior of spiders will readily supply additional difficulties.

The first series, undertaken with *Acrosoma rugosa* only, was an attempt to determine directly the relation of various factors to the height at which webs of the species are built. In a preliminary experiment ten spiders were placed in a small screen cage 45 cm high and left over night on a table in the laboratory. The next morning all had built webs, seven of them within two inches of the top, and the other three in the upper half. The cage was then reversed and one hour later all individuals had begun webs in the upper half of the cage.

In Experiment 7-19 (Table 1) ten individuals were placed under inverted battery jars 33 cm by 45 cm high on a laboratory table. Strips of corrugated paper were supplied for foot-hold, and the upper two-thirds of one jar was covered with black paper. Observations were then made every two minutes and the positions of the spiders recorded. The spiders consistently sought the upper part of each cage, regardless of the partial darkness in the one. Light does not seem to be a determining factor.

Forty-four spiders were placed in a 6-foot screen cage in the dark-room late in the afternoon. The next morning thirty were between 5 and 6 feet from the floor, nine between 2 and 3 feet from the bottom, and five on or near the bottom of the cage. The wooden crossbars at the bottom, at 3 feet, and at the top afforded good points for attachment of webs. This experiment indicates that the selection of the position for the web takes place almost, if not quite as well in darkness as in light, and is largely governed by architectural conditions.

TABLE I

Experiment No. 7-19. Showing the reactions of the spider *Acrosoma rugosa* Hentz, in a vertical light gradient.*

Minutes from	Control			Experiment		
3:40 P.M.	*1*	*2*	*3*	*1*	*2*	*3*
0	5	0	0	5	*0*	*0*
10	0	0	5	1		4†
20	0	2	3	1		4
30	0	1	4	0		5
60	0	1	4	0		5
120	0	1	4	0	0	5

* Five spiders were placed in each of two inverted glass battery jars 45×33 cm, one of which (experiment) was covered with black paper on the top and the upper two-thirds of the sides. The other was uncovered, and both stood on a laboratory table receiving light from a North window 3 M. distant. The spiders had been brought from the field the previous day. Temperature of the room was 80° F.

In the above record, the numbers in the columns 1, 2, and 3 represent, respectively, the number of spiders in the corresponding third of the cylinder (beginning from the bottom) after the time-interval designated in Column 1.

† On account of the covering, separate observations could not be made in the upper two-thirds of the cylinder except at the beginning and end of the experimental period.

TABLE 2

Summary of experiments on the reactions of spiders in gradients of the evaporating power of air.

Species	Exp. No.	Wet	Medium	Dry
Acrosoma rugosa	Evap.	0.0–0.8	0.2–1.4	2.2–2.8*
	1	59	43	138
	2	44	83	113
	3	74	45	121

	3a	56	44	140
	7	103	124	173
	7a	99	39	262
Totals		435	378	947†
Percent		24.7	21.5	53.8
Epeira gibberosa	Evap.	1.1–1.3	1.8–2.1	2.7–3.0*
	92	92	67	1
	92a	64	57	39
	92b	80	98	42
	92c	73	106	41
Totals		309	328	123†
Percent		40.7	43.1	16.2
Anyphaena	Evap.	1.8	2.8	3.7*
	12	23	15	28
	12a	71	14	2
Totals		94	29	30†
Percent		61.4	18.9	19.7
Dendryphantes aestivalis	Evap.	1.8	2.8	3.7
	11	48	19	53
	11a	38	19	63
	11b	35	17	68
Totals		121	55	184†
Percent		33.6	15.3	51.1

* Evaporation in cc. per hour.

† Totals of readings at one-minute intervals through the entire experiment.

TABLE 3

Summary of experiments on the reactions of spiders in gradients of air temperature.

Species	Exp. No.	1.	2.	3.
Acrosoma rugosa	Temp.	23.5°	26.0°	27.5°
	10	181	72	47*
	Percent	60.3	24.0	15.7
	Temp.	26.0°	27.0°	28.5°
	10a	179	94	27
	Percent	59.7	31.3	9.0
	Temp.	29.0°	33.0°	35.0°
	9	194	71	35
	Percent	64.6	23.7	11.7
	Temp.	33°	35°	36°
	9a	177	36	87
	Percent	59.0	12.0	29.0
Anyphaena	Temp.	20°	25°	29°
	103	119	44	77
	103a	134	1	105
	Total	253	45	182
	Percent	52.7	9.3	38

Dendryphantes aestivalis	Temp.	25°	30°	37°
	101	95	20	25
	Percent	79.2	16.7	4.1
	Temp.	25°	26°	28°
	101a	67	15	38
	Percent	55.8	12.5	31.7

* Totals of readings at one-minute intervals through entire experiment.

TABLE 4

Experiment 8. Showing the reactions of *Acrosoma rugosa* Hentz in a vertical gradient of the evaporating power of air.

	Experiment 8		
	Bottom Section	Middle Section	Top Section
Evap.	2.5cc	1.4cc	0.7cc
Minutes			
0	8	0	0
10	3	2	3
20	1	3	4
30	1	2	5
40	2	1	5
Total	87	83	150

(Readings at one-minute intervals)

Eight animals were placed in the lower third of the same cage used in previous experiments, set on end, and observations of their positions were recorded at one-minute intervals. The evaporating power of the air expressed in cubic centimeters of water evaporated from a standard atmometer in one hour is indicated at the head of each column.

Observations in the field showed that the most usual situation for a web of *A. rugosa* is a leafless shrub with lateral branches near enough to another point of attachment to make possible the characteristic structure of the web. If the dead shrub supplies a suitable point of departure the spider may swing to the other side and carry across the first line of the web, otherwise a thread is spun which is carried by an air current across the intervening space and becomes attached because of its viscous nature. Spiders often attempt to establish webs where there is no possibility of success by either method. Fruitless attempts several hours in duration may be made to establish a web from a seemingly suitable point of departure, and free lines several meters in length may be spun in the attempt to find a second point of attachment.

Additional experiments with this species included the observation of the reactions of the species in gradients of the evaporating power of air and temperature. Experiments 1, 2, 3, 3a, 7, and 7a illustrate the former, and Experiments 9, 9a, 10 and 10a are examples of the latter. Results of these experiments are recorded in Tables 2 and 3. In these experiments the cages and air conditioning apparatus described by Shelford and Deere

(1913) and Shelford (1914) and previously used by the author (1917, 1919) were utilized.

The tabulated results of these experiments show a strong tendency for the animals to seek the driest third of the cage, even when the evaporation amounts to as much as 2.8 cc per hour. This is an evaporating power much greater than that ordinarily encountered in the normal habitat. The maximum mean hourly evaporation observed for a one-week period was a little over 1 cc per hour, at the forest margin in the middle of July. Assuming, on the basis of observational data, that three-fourths of this evaporation took place in the day time, we obtain an hourly mean for daylight hours of about 1.5 cc. The spider concerned is never found in this area. Figured in the same way, the maximum hourly evaporation in the shrub stratum where this species is found could not have exceeded 1.25 cc. with the usual value much below this. The explanation of the experimental results is somewhat difficult. The only suggestion that offers itself is that the animal selects the place of highest evaporation available where other conditions are favorable. The evaporation at 1.25 m, thea verage height of the web of this species, is much greater than at ground level.

Summaries of experiments involving a gradient of air temperature are given in Table 3. Here the results indicate an optimum temperature of 23.5° or lower. As the lowest temperatures are found near the ground level, this factor would tend to regulate the height to which the spiders ascend but the natural vertical gradients of neither temperature nor evaporation are enough to determine the location of the webs. In fact, as shown in Table 4, which records the results of an experiment involving a vertical evaporation gradient with the low evaporation in the upper part of the cage, the spiders seek a higher level even if in so doing they pass into a region of lower evaporation. Whether a difference in evaporation would determine the height to which the animal would ascend, within the limits available in the field, was of course, a question impossible of solution with the small cages, etc., available.

The tendency to seek higher levels is, within certain limits, at least, in this species independent of the evaporating power and presumably of the temperature of air. While the animal reaches and builds its web at a level at which there is a certain balance between the optima selected in the laboratory, this height seems to be determined by mechanical and structural relations rather than by the direct effects of the conditions of the air.

Epeira gibberosa, which occurs at or near the top of the herb stratum at an average height of .7 m was also subjected to a gradient of the evaporating power of air. Experiments 92, a, b, and c, which are recorded in Table 2 show the reactions of this spider. In contrast with *Acrosoma rugosa* this species showed a preference for a region of less intense evaporation, averaging about 1.8 cc per hour, a condition somewhat approximating the maximum evaporation at the level mentioned in the more open areas of the woods. Field experiments also showed that this species exhibits a strong positive phototropism. Other experiments were not successful because of the rather small size of the animal and the ease with which it is injured. The experiments just outlined give results which accord very closely with what one might expect from a knowledge of the habitat. The spider is found near the tops of the herbs, in the more open parts of the woods, often in full sunlight, in a region of a comparatively high evaporation.

Immature specimens of *Anyphaena* sp. found at an average height of 1.25 m, often in curled leaves or in similar sheltered places, protected by a silken tube, were also subjected to gradients in temperature and evaporating power of air. Summaries of experiments 12, 12a, 103 and 103a, Tables 2 and 3, illustrate the behavior of this species. The optimum temperature seems to be in the neighborhood of 20° C, and the most favorable evaporation at about 1.7 cc or less, per hour. The normal evaporation in a rolled leaf, and with the protection afforded by a silken tube, is, of course, much less than in an openly exposed web at the same height, where the maximum is probably somewhat greater than the value just given. The lowest temperature available was chosen as in the case of Acrosoma.

Dendryphantes aestivalis is a wandering spider inhabiting especially low shrubs and small trees. On account of the lack of a web or definite retreat it is less restricted in its normal daily movements than any of the forms previously discussed. In a gradient of the evaporating power of air, (Table 2), while there was a preference for the drier parts of the cage with an evaporating power of 3.7 cc per hour, this preference was not marked. The larger figures for the terminal portions of the cage may be explained by the tendency of the spiders to occupy the corners.

In a gradient of air temperature, as illustrated by Experiments 101 and 101a (Table 3) *D. aestivalis* also showed a preference for the lowest temperature available. This preference was very decided when a difference of 12° between the ends of the cage was maintained and less marked when this difference was only 3°.

Temperatures much below the outdoor summer temperature could not be maintained, with a flow of air, in the apparatus, but in each case the temperature chosen by the spiders used in the experiments was much below that prevalent in the open fields. It is probable that this temperature reaction is one of the important factors limiting the distribution, horizontally, and perhaps vertically of the species considered. Evaporation seems to be of lesser importance as the spiders show a preference for an evaporation much higher than that encountered in their natural habitats. The antagonistic action of the two stimuli may be partially responsible for the phenomena of vertical distribution of spiders, but it appears that the physical environment afforded by plant structures is of considerably greater importance in this respect.

2. *Effect of Environmental Conditions on the Rate of Development.* In order to test the effect of temperature and humidity conditions on the time elapsing until emergence, cocoons of *Epeira gibberosa* Hentz, were gathered at various times during the autumn and winter, brought to the laboratory and kept under controlled conditions. Each cocoon was placed in a short piece of glass tubing of about 5 mm inside diameter and about 3.5 cm long, the ends of which were closed by a loose plug of cotton. The tubes were then fastened to strips of wood in groups of ten, for the sake of easier handling, and placed in the controlled experimental cages. A complete series involving contrasting humidities at all temperatures used, and vice versa, was not available, but an attempt was made to obtain widely variant conditions. Records of the conditions in the various cages were made with the aid of recording thermographs and hygrographs, and evaporation was, in most cases, determined by the use of the porous cup atmometer. The cages BH, BW, AL and ALL (See Table 7) were of glass and sheet metal over an earth-filled flowerpot, similar in every way, and the air was allowed to flow through all at the same rate. CLL was a small glass cage, while HI was an unlighted refrigeration chamber. Both were adequately ventilated, but the rate of flow of the air could not be compared with that in the other cages. All cages except HI were illuminated by daylight (passing through several thicknesses of glass) and AL and ALL were, in addition, during the day, illuminated by large nitrogen-filled daylight lamps. B was the large constant-temperature room in which cages BH, BW and CLL were located. Temperatures in this room were approximately constant, except for a noonday rise of from three to five degrees during the last six weeks of the experimental period. Cages AL and ALL were in an adjoining room, the temperature of which was variable, simulating a simplified ideal winter day. All figures given in this table represent the means of weekly data.

As the cages could not be opened without changing, for the time being, the conditions (especially the humidity) it was desired to maintain, the cocoons were examined but once a week, at the same time that it was necessary to open the cages to change the record sheets on the instruments. Although an error was introduced into the calculations in this way, it is felt that the disadvantages of this procedure were not as great as those attending a more frequent disturbance of conditions.

Control lots of cocoons were placed in glass tubes in the same manner and exposed to outdoor conditions in the screen-house adjoining the room in which the controlled apparatus was located. In some cases cocoons were changed from one experimental chamber to another. A considerable number were from time to time transferred from less favorable conditions to the high temperature and high humidity of cage BH, or to BW, where the humidity was somewhat less.

It was assumed that the processes of development ending in emergence proceeded at a uniform rate under constant conditions, and that when a cocoon was transferred from one set of conditions to another the amount of development taking place in each was proportional to the product of the rate of development for that set of conditions by the time under those conditions. On this basis velocity factors were computed for each experimental cage. An example will suffice to illustrate the method. Comparing spider lots Aa and Ae (Table 5) we find that the first was left in cage HI for 69 days and then transferred to cage BH where 21 days elapsed before emergence. Lot Ae remained in cage BH an average time of 67 days before emergence. Assuming that both lots were at the same developmental stage at the beginning of the experiment, we may derive the following algebraic equation from the data just given:

$$69V_{HI}+21\ V_{HB}=67\ V_{BH},$$

where V_{HI} and V_{BH} represent the velocity factors for cages HI and BH, respectively, hence:

$$V=_{BI}1.5\ \ V_{HH}$$

Taking the velocity factor of HI as unity, we obtain the relative value of 1.5 for V_{BH}. Similarly, by comparing lots Ca and Cb, we obtain a value of 1.7. Further comparisons led to the adoption of 1.6 as an average value for V_{BH}, when compared to V_{HI} as 1.0. Similar calculations were made for the other sets of conditions, and the velocity factors given in Table 5 were obtained. The sums of the products of these factors by the number of days in the corresponding cages gave values approximately constant within each group of cocoons brought from the field on the same day. The further assumption was then made that all were in the same stage of development on October 15, and the number was obtained, which, when multiplied by the number of days elapsing between October 15 and the beginning of the experiment, and added to the number just mentioned, gave a number approaching a constant for the entire experiment. The figure thus obtained (0.65 for Epeira and 0.85 for Arachnophaga) represents the velocity factor for outdoor conditions. This does not represent, as do the other velocity factors, the relative velocity of development under a stable set of conditions, but is rather a summation of many velocity factors operating through the season. None of the velocity factors obtained in this manner should be considered as definite and exact quantities, but rather as a means by which certain relationships can be brought to the attention. The real velocity factor for a certain set of conditions would depend upon the stage of development already reached by the organism and its previous physiological history. While the limitations of this method are rather obvious, it is perhaps almost equally evident that it is not limited in its application to the estimation of the comparative developmental value of experimentally controlled conditions. If we have definite knowledge as to the climatic conditions prevailing during a series of seasons, we may tentatively develop a velocity factor for each combination of conditions. Phenological predictions made on the basis of such calculations would undoubtedly be more likely of fulfilment than those made on the basis of temperature cumulations only, or than those based on any other single factor.

Table 5 summarizes the data obtained as to the time of emergence of the spiders under the different experimental conditions. It will be seen that the most rapid development took place in Chamber BH, with a mean temperature of 25.8° and with the air saturated with moisture. The rate in Chamber HI with a mean temperature of 18° and a mean relative humidity of 64 per cent was about six-tenths of this, provided the cocoons were removed to BH after a period of about fifty days. If retained longer at the lower temperature (and lower humidity) the true value of the factor would be much less, as is shown by the high value of the "constant" obtained in lots Df and Fd. These results indicate that development proceeded, up to a certain point, at the low temperature and low humidity of this cage, but that a limit was reached beyond which development was very slow, with the probability of the occurrence of death in a short time. In fact, as will be seen from Table 6, no spiders emerged unaided from cocoons kept in HI, and large numbers of well developed spiderlings were found dead in the cocoons in this cage. No spiders were able to emerge except in air of very high humidity.

Cage AL gave a velocity factor about two-thirds that of HI, with the difference that emergence was possible under the conditions prevailing here. The day temperature in AL reached an average of 21.2° while the night temperature fell, on an average, to 1.7° with a mean of 9.3°. The humidity averaged above 90 per cent. On account of the slowness of the processes of development here not all of the spiders had emerged when the experiment closed, but even at this low temperature complete development was possible. The threshold of development is evidently very low. No preliminary period of freezing was necessary for emergence, but the fact that the velocity factor for outdoor conditions seems to have nearly the same value throughout the winter suggests a stimulating effect of low temperature or of variable temperatures. Further data are necessary for the determination of the threshold of development and the point to which development may proceed at low temperatures without a high moisture content. The short time required for the emergence of the spiders from the single productive cocoon of lot De is unexplained. This cocoon may have been formed several weeks earlier than the others.

The percentage of cocoons producing living spiders was about the same under the conditions prevailing in cages BH and AL, and in the lots placed first in HI and then removed to BH. The highest percentage of spider-producing cocoons was found in the control lot, Bh (Table 6). In this lot there were no dead spiders which could be identified as such when the cocoons were opened, and fifty per cent of the cocoons had either produced living spiders or still contained them at the end of the experiment.

A summary of the data from all experiments shows that the number of cocoons containing spiders, living or dead, totalled 48 per cent of the entire number, and of 52 cocoons examined on January 9, exactly half contained living spiders while the remainder were parasitized. Thus the mortality in unparasitized cocoons may be considered as zero in the control set.

None of the experimental conditions were as favorable for normal development as were outdoor conditions, although development took place more rapidly in several of the former. It will also be seen that the outdoor conditions were much more unfavorable for the development of the parasites. No spiders were able to emerge, in the experimental cages, where the relative humidity averaged less than 90 per cent, even under the most favorable temperature conditions, and at high humidity spiders were able to complete their development at a very low temperature. A variable temperature, or a period of medium temperature followed by high temperature with high humidity, seems to be more favorable than a prolonged high temperature, although the last mentioned condition produced the most rapid development. The first two conditions most closely approach those of the natural habitat.

It was evident that the hymenopterous insect parasitic on the eggs of this species (*Arachnophaga picea* Riley) was able to reach maturity and to emerge under much more varied conditions than those favorable for the development and emergence of the spiders. Records of the emergence of these insects were made in the same way as in the case of the spiders, and the same procedure was followed in obtaining velocity factors and in computing "constants." The data thus obtained are also given in Table 5.

The most rapid development took place in lot Ba, which was brought from the field on November 8 and placed in cage HI for two months, after which it was removed to cage BH. The value of the "constant" in this case is conspicuously low, and differs markedly from those computed from the data furnished by lots Aa, Cb and Dd, which were given the same treatment in the same sequence. The only difference between lots Aa and Ba was the length of time under outdoor conditions, as the latter was brought to the laboratory eight days after the former. Before October 31, the temperature had not fallen below the freezing point of water, but a heavy frost, with a minimum temperature of −3.3° occurred the morning of November 2. As the second lot developed so much more rapidly than the first, we must conclude that freezing was the cause of the variation. It appears, then, that the most rapid development of this insect takes place if it is subjected to freezing temperature for a short time, then to a moderate temperature for a considerable period, and finally, to a high temperature. The fact that development took place in about the same time in CLL (dry) and BH (wet) seems to indicate that, at high temperatures at least, humidity is not of great importance. The "constants" obtained from lots Cb and Dd were again of the more usual magnitude, indicating that further freezing did not increase the velocity of development. However, the stimulating effect of low temperature or of variation was such that throughout the series the velocity factor for outdoor exposure was nearly as large as that for cage HI with a much higher mean temperature. It is also evident that humidity was a more important factor at the lower temperatures.

Insects also developed more rapidly when subjected to a moderate temperature for a time after being brought from the field, than if introduced directly to a high temperature. This will be seen by comparing the data from lots Ba and Bb, Bc and Bd. Of these the second was introduced immediately into cage BH (high temperature, high humidity), the third was introduced first into CLL (low humidity) and then into BH, while the fourth was left in CLL. All developed much more slowly than the first, which remained for two months at the lower temperature before being subjected to the high temperature and high humidity. There seemed to be no difference between the CLL-BH combination and direct introduction into BH, while the cocoons left in CLL required an additional week.

The Time X Velocity Factor product was highest for Series A, E, and F. The higher value for the first group may be explained by assuming that without a preliminary freezing, the processes of development require a longer time. Series E and F gave high values because a longer time at low temperature did not further accelerate development.

The highest proportion of *Arachnophaga picea* developed from the cocoons of lot Da which were brought from the field on January 16 and immediately placed in the high temperature-moderate humidity chamber. Over 25 per cent of the cocoons of this lot yielded parasites, while the proportion among all cocoons in this chamber was nearly as great. This high proportion was also obtained from a CLL-BH combination, at the same temperature. HI alone and BH alone were very unfavorable. It was rather remarkable that no parasites were found in the control series subjected to outdoor conditions. The parasites in that lot evidently perished in the larval stage, as no exoskeletal remains were observed. Numbers of dead imagos were found in the HI series and none emerged unaided from cocoons retained in this environment throughout the experimental period. The combination of low humidity and medium temperature in this chamber seemed to allow development up to a certain point beyond which a change of some sort was necessary to avoid death. This could not have been due entirely to low temperature, as several insects emerged in each of the variable low temperature cages. In both of these, however, the moisture content of the air was greater than in HI. Moisture seems to be of little importance for the development of Arachnophaga at high temperatures, in fact saturated air seems to be less favorable for rapid development than air with a lower moisture content, but at low temperatures moisture becomes a limiting factor.

In only a few cases did both spiders and parasites develop in the same lot of cocoons. The lots concerned were Aa and Dd, Ab, and Bc. The two first mentioned were subjected to the HI-BH combination, medium temperature-low humidity followed by high temperature-high humidity, and in these the spiders completed their development in less time than the parasites. The remaining two were from cage AL, low temperature and high humidity. Here the parasites developed first, much sooner in the case of Bc which had been frozen before being brought to the laboratory. The parasites are able to complete their development in a dry environment, while the spiders can not emerge unless the humidity is above 90 per cent. While low and variable temperatures stimulate the development of both forms, this stimulus is more prominent in the case of the parasite, which also has a lower threshold of development.

TABLE 5

Length of time to emergence of spiders and parasites (*Arachnophaga picea*) from cocoons of *Epeira gibberosa* under various conditions.

Epeira gibberosa

Lot	Date Begun	Number of productive cocoons	Time in days in cages			Total number of days x velocity factor	Average
			1*	2	3		
Aa	Oct. 31	1	O 16	HI 69	BH 21	113.2	
Ab		2	O 16	AL 169		120.3	
Ae		2	O 16	BH 67		118.3	118.1
Be	Nov. 8	4	O 24	AL 165		122.9	
Bg		2	O 24	BH 56.5		106.6	117.5
Bi	Nov. 8	10	Control			126.8	126.8

* The number in this column indicates the number of days between October 15 and the date on which the experiment was begun.

Lot	Date Begun	Number of productive cocoons	Time in days in cages			Total number of days x velocity factor.	Average
			1*	2	3		
Ca	Jan. 9	2	O 86	BH 41		121.9	
Cb		5	O 86	HI 48	BH 14	126.4	
Cc		4	O 86	AL 90		114.4	121.2
Dd	Jan. 16	6	O 93	HI 41	BH 11.7	120.3	
De		1	O 93	BH 13		81.4	
Df		1	O 93	HI 92		152.5	119.4
Eb	Jan. 30	2	O 108	R† 10	BH 17	112.6	112.6
Fd	Feb. 6	1	O 114	HI 68		142.1	142.1
			Arachnophaga picea				
Aa	Oct. 31	2	O 16	HI 69	BH 56	131.7	
Ab		2	O 16	AL 146.5		120.0	
Ac		1	O 16	CLL 124		125.2	
Ad		1	O 16	ALL 169		131.8	126.7
Ba	Nov. 8	4	O 24	HI 61	BH 26.2	101.5	
Bb		1	O 24	BH 96		116.4	
Bc		2	O 24	CLL 61	BH 35	110.3	
Bd		3	O 24	CLL 103		113.1	
Be		2	O 24	AL 120.5		116.8	
Bf		1	O 24	ALL 138		117.0	110.2
Ca	Jan. 9	1	O 86	BH 34		107.1	
Cb		1	O 86	HI 48	BH 14	130.3	118.9
Da	Jan. 16	11	O 93	BW 39.7		118.8	
Db		2	O 93	B 34		110.1	
Dc		1	O 93	CLL 34		110.1	
Dd		1	O 93	HI 48	BH 14	136.2	118.2
Ea	Jan. 30	1	O 106	R 10	BW 24	122.6	122.6
Fa	Feb. 6	3	O 113	R 3	BH 21.7	120.3	
Fb		2	O 113	R 3	BW 24	122.6	
Fc		2	O 113	R 3	B 24	122.6	121.6

Velocity factors used in above table	AL	ALL	B	BH	BW	CLL	HI	O	R
Epeira	0.65			1.60			1.0	0.65	1.5
Arachnophaga	0.80	0.70	0.90	1.00	1.00	0.90	0.90	0.85	0.85

† The letter "R" indicates that the cocoons were kept in the laboratory for the number of days indicated in the next column.

TABLE 6

Viability of the spider *Epeira gibberosa* Hentz and its hymenopterous parasite *Arachnophaga picea* Riley under various conditions.

Lot	Cages		Dates			Numbers					
	1	2	begin.	Trans.	End.	S	P	DS	DP	E	Egg
Ae	BH		Oct. 31		Apr. 18	2	0	2		6	
Bb	BH		Nov. 8		Apr. 18	2	1	5		12	
Ca	BH		Jan. 9		Apr. 18	2	1	3		4	
De	BH		Jan. 16		Apr. 18	1	0	1		8	
Eb	R	BH	Jan. 30	Feb. 9	Apr. 18	4	1	0		5	
						11	3	11		35	
						16%	5%	16%		58%	
Aa	HI	BH	Oct. 31	Jan. 8	Apr. 18	1	2	4		3	
Ba	HI	BH	Nov. 8	Jan. 8	Apr. 18	0	4	2		4	
Cb	HI	BH	Jan. 9	Feb. 26	Apr. 18	5	1	6		8	
Dd	HI	BH	Jan. 16	Feb. 26	Apr. 18	6	1	0		3	
						12	8	12		18	
						24%	16%	24%		36%	
Af	HI		Oct. 31		Apr. 18	0	0	7	3	8	2
Bh	HI		Nov. 8		Apr. 18	0	1*	4	3	2	0
Cd	HI		Jan. 9		Apr. 18	0	0	4	1	5	0
Df	HI		Jan 16		Apr. 18	1*	0	8	0	1	0
Fd	R	HI	Feb. 6	Feb. 9	Apr. 18	1*	0	6	1	1	1
						2	1	29	8	17	3
						3%	1.5%	48%	13%	28%	4.5%
Da	BW		Jan. 16		Apr. 18	0	11	19	0	10	
Ea	R	BW	Jan. 30	Feb. 9	Apr. 18	0	1	5	0	4	
Fb	R	BW	Feb. 6	Feb. 9	Apr. 18	0	3	6	0	1	
						0	15	30	0	15	
						0%	25%	50%	0%	25%	
Ab	AL		Oct. 31		Apr. 18	2	1	11	0	6	0
Be	AL		Nov. 8		Apr. 18	4	3	1	1	1	0
Cc	AL		Jan. 9		Apr. 18	3	0	0	1	5	1
						9	4	12	2	12	1
						22%	10%	30%	5%	30%	2.5%

Lot	Cages		Dates begin.	Trans.	End.	Numbers					Egg
	1	2				S	P	DS	DP	E	
Ad	ALL		Oct. 31		Apr. 18	0	1	4	1	4	0
Bf	ALL		Nov. 8		Apr. 7	0	1	3	2	3	1
						0 0%	2 10%	7 35%	3 15%	7 35%	1 5%
Ac	CLL		Oct. 31		Apr. 18	0	1	6	0	3	0
Bd	CLL		Nov. 8		Apr. 18	0	5	7	0	7	1
Cd	CLL		Jan. 9		Apr. 18	0	0	10	0	10	0
Dc	CLL		Jan. 16		Apr. 18	0	1	5	0	4	0
						0 0%	7 11 %	28 46%	0 0%	24 40%	1 1.6%
Bc	CLL	BH	Nov. 8	Jan. 8	Apr. 18	0	5	7	0	7	1
Db	B		Jan. 16		Apr. 18	0	2	0	0	2	
Fc	R	B	Feb. 6	Feb. 9	Apr. 18	0	0	8	1	2	
						0 0%	2 13%	8 53%	1 6%	4 26%	
Bi	Control		Nov. 8		Apr. 18	10 50%	0 0%	0 0%	0 0%	10 50%	

Explanation of table:

Column headings.

Numbers:—

Number of cocoons yielding living spiders. In this and the next column the (*) indicates living animals not yet emerged from the cocoon on April 18.

P:—Number of cocoons yielding living parasites.

DS:—Number of cocoons containing dead spiders on April 18.

DP:—Number of cocoons containing dead parasites on April 18.

E:—Number of cocoons empty or containing remains which could not be positively identified.

Egg:—Number of cocoons containing unhatched spider eggs.

TABLE 7

Conditions in experimental cages referred to in Tables 5 and 6.

Cage	Temperature (Degrees C)			Relative Humidity (Percent.)			Evap. Power of Air. (cc. per hour)
	Max.	Min.	Mean	Max.	Min.	Mean	Mean
BH	30.0	21.0	25.8	100%		100%	0.04
HI	19.5	16.5	18.0	69.5	56.7	63.9	0.52
BW	30.0	21.0	25.8	92.8	68.0	83.9	0.78
AL	21.2	1.7	9.3	98.2	86.8	93.6	
ALL	21.2	1.7	9.3	97.0	57.7	78.2	
CLL	30.0	21.0	25.8				1.00
B	30.0	21.0	25.8				1.00

SUMMARY OF BIOLOGICAL CONCLUSIONS

1. Random sampling of the upper soil, leaf, herb and shrub strata in the elm-maple forest showed variations of the animal population in general in inverse ratio to variations in the evaporating power of the air. This is the reverse from conditions in the pine dune community as found by Sanders and Shelford (1922).

2. A great and sudden increase in the insect population as determined by random sampling occurred in the early autumn, the maximum collections being made on October 3. This was due to the autumnal migration of hibernating species from the forest border and the adjacent meadows, which was associated with the gradual decline in temperature and an increase in the daily mean variability due to lower night temperatures. The inward migration occurred at the level of the normal summer habitat of the species concerned and was followed by a downward migration to the place of hibernation.

3. A similar increase, marking a migration in the opposite direction, was indicated by the character of the spring collections.

4. Of non-migratory animals especial attention was given to the spiders, which may be divided into three groups according to their manner of adjustment of life history to the annual rhythm of the deciduous forest. Most species pass the winter in hibernation in the adolescent state. The second group spends the winter in the egg case, hatching either in late autumn or early spring, while the third differs from the first only in a greater degree of activity during the winter.

5. The animal association of the elm-maple forest is characterized by the appearance of marked seasonal societies, those of spring and autumn being dominated, especially, by the migrant forms.

6. On the basis of interchange of societal subdominants between prairie and forest it is held that the savanna constitutes a unit animal community.

7. Experiments involving the reactions of spiders in gradients of the various environmental factors differing at the different levels in the forest indicate that these factors are probably of considerable importance in determining the horizontal and vertical distribution of the animals but that their relative importance is not the same for different species. Among web-building species the mechanical features of the environment as related to the support of the web are also of very great importance.

8. Cocoons of *Epeira gibberosa* Hentz, some of which were parasitized by *Arachnophaga picea* Riley were subjected to controlled atmospheric conditions, with results leading to the following conclusions:

a) High temperature (mean 25.8° C) and a relative humidity near the saturation point caused most rapid development of the spiders, but mortality was lowest under outdoor conditions.

b) Spiders were unable to complete development or to emerge except under conditions of high humidity.

c) The parasites developed most rapidly when allowed to remain in the open until after the occurrence of freezing temperatures, then placed for two months in a low temperature-low humidity chamber (means 18.0°C and 63.9 per cent), and finally removed to a high temperature (mean 25.8° C) high humidity chamber. The humidity at this temperature seemed to have the effect of increasing the mortality, however, while a lower humidity (mean 83.9 per cent) at the same temperature produced just as rapid development.

d) Mortality of the parasites was lowest when the cocoons were kept for the greater part of the time, at least, at a high temperature and a moderate or low humidity, and greatest under outdoor conditions.

e) The threshold of development of the parasite was found to be lower than that of the host. The host developed more rapidly, however, at high temperatures.

f) Relative velocity factors for each set of conditions were computed and the law that the summation of the products of the velocity factors by time gives a constant, was developed. This relation may be expressed as follows:

$$(T_1V_1+T_2V_2+\ldots\ldots\ldots+T_nV_n)=K$$

where T_1, T_2, etc. represent the length of time spent under each set of conditions, and V_1, V_2, etc., represent the corresponding velocity factors. When sufficient data are available such velocity factors may be computed for any set of conditions, and for any developmental process influenced by such conditions. Results thus obtained may be utilized in phenological predictions.

9. Data obtained as indicated above have a definite relation to the adjustment of the life cycles of the animals considered to the annual climatic rhythm of the temperate deciduous forest and savanna.

SUMMARY OF METEOROLOGICAL OBSERVATIONS

1. *Humidity*—Recording hygrographs, standardized each week, were used in obtaining humidity data. Two instruments were used, one (a hygrothermograph) in a shelter about 100 m from the western edge of the forest in McDougall's (1922) quadrat No. 76. The sensitive element of this instrument was about 60 cm above the surface of the ground. The other hygrograph was suspended about 10 m above the ground in a maple tree in quadrat No. 54.

The record sheets were ruled off into two-hour intervals, and this period was taken as the unit. The mean humidity for each period was estimated, the average of these means (Monday to Monday) being computed as the weekly mean humidity. The daily maxima and minima were also averaged and the daily drop in humidity below the preceding maximum and below the preceding base mean was computed. The latter term is defined as the mean relative humidity between the hours of 8 p.m. and 6 a.m. This period was chosen because it is a period of relatively uniform humidity and temperature, or at least the period of most uniform conditions.

The relative humidity curve shows a very low point during the third week (week ending July 18) and four high points, during the weeks ending September 5, November 28, March 13, and May 29 (Fig. 2).

Examination of the variation curves shows that, in general, a wide range accompanies a low mean relative humidity. This is because of the fact that the moisture content of the air rarely fails to reach saturation during the night hours, and a low mean relative humidity is almost always caused by a very low day-time value.

A comparison of the data from the station between the herb and shrub strata with those from the tree station shows an almost invariably greater mean relative humidity in the former situation and a greater mean daily range in the latter. There were three slight exceptions to the latter statement, and four cases in which there was no difference in the mean at the two stations. The average difference for the entire period of observation at both levels was 3.5 per cent.

Lorenz-Liburnau's data show the following differences of relative humidity between the ground level and a height of 11 m, in beech woods:

Time	Relative Humidity at 0 m. Minus Relative Humidity at 11 m.
Forenoon (6:17-11:00 a.m.)	5.8%
Mid-day (11:00-3:00 p.m.)	10.9
Afternoon (3:00-6:18 p.m.)	8.0

The greatest differences were observed between the ground and a height of 5 m. Data obtained by the use of atmometers, as will be shown later, would place the zone of the most rapid decrease in humidity within a meter of the surface of the ground. Lorenz-Liburnau obtained, as an average of 79 observations between May 23 and October 16, a difference of 7.73 per cent between the relative humidity at the soil surface and that at 11 m. His method, however, left entirely out of consideration the hours between 9 p.m. and 4:30 a.m.

The hygrograph records for the week ending September 12 were analyzed and the means for each two-hour period were obtained, with the following results:

Hours	Mean R. H. at 0.6 m.	Mean R. H. at 10 m.	Difference
6-8 a.m.	95.0%	95.0%	0.0%
8-10	90.0	84.3	5.7
10-12 m.	77.1	76.0	1.1
12-2 p.m.	77.0	71.3	5 7
2-4	80.5	70.3	10 2
4-6	86.0	73.7	12.3
6-8	88.5	81.8	6.7
8-10	92.8	87.5	5.3
10-12 n.	93.5	91.8	1.7
12-2 a.m.	93.7	92.0	1.7
2-4	94.0	93.0	1.0
4-6	94.8	95.3	— 0.5
		Average Difference	4.3

See Figure 6 for a graphic representation of the temperature and humidity variations during the "ideal day." The hygrothermograph records for the week are given in Figure 7. The low average difference, compared with that obtained by Lorenz-Liburnau, may be explained by the fact that his observations left out of consideration those hours when the humidity differs least at the various levels, and the fact that the lower level used in the present observations was not ground level but 60 cm above.

Relative humidity of the air is greatest at ground level in the forest and decreases upward, very rapidly at first and then more slowly. During early morning hours or during fogs, rains, etc.,there may be no gradient. The temperature relation is such that, even with the same amount of absolute humidity at the different levels, a gradient in this direction would be present. However, the gradient, especially near the ground, is too abrupt to be caused by the slight temperature differences alone, and there is a gradient in absolute as well as relative humidity.

2. *Evaporating Power of Air.*—The use of the evaporating power of the air, determined by the porous cup atmometer, as a summation of the effects of temperature, humidity, air movement, insolation, etc., has been too well established by various investigators to require discussion here. During the season favorable for the use of the porous cup atmometer, i.e., during the season without temperatures below the freezing point of water, these instruments were exposed in twelve different places in and at the borders of the woods. Although observations at all of the stations were not begun on the same date, and although there were some interruptions due to accident, a fairly representative series of readings was obtained.

The atmometers were mostly new and had been standardized by the manufacturers. All were re-standardized after use, and in some cases more frequently. They were mounted in the customary manner, without rain correcting devices. Those in the trees were protected by a cylindrical guard of coarse wire netting.

Atmometers were exposed at the following stations:

1.—On the top of the instrument shelter 1 m above the surface of the ground, surrounded by large elm and maple trees and by lower trees and shrubs, such as ironwood, white ash, buck-eye, red oak, spice-bush, red-bud, etc.

2.—About 3 m from the instrument shelter, on the ground, under cover of a thicket of Benzoin. The porous cup was about 10 cm above the surface of the ground.

3.—At a height of 1.5 m on the south side of the trunk of an ash tree, 25 cm in diameter, about 1 m from the instrument shelter.

4.—At the same height, on the north side of the same tree trunk.

5.—At a height of 2.5 m, suspended under a leafy branch of a small hard maple, about 3 m from the instrument shelter.

6.—In a hollow stump about 20 m from the instrument shelter. The heart of the stump was decayed so that the cavity reached down to ground level. The cortex was also broken away on the southeast side leaving an opening about 20 cm in width.

7.—At the west edge of the woods in the short grass near the roadside. This atmometer was stolen during the week ending August 1, and observations at this station were discontinued.

8.—At a height of 6 m in an elm tree about 10 m from the instrument shelter.

9.—At a height of 10 m in a maple tree about 50 m from the instrument shelter in a group of large maple and elm trees.

10.—This instrument was first exposed at a height of 12 m at the top of the same tree as No. 8. After September 13 it was removed to the top of a buck-eye midway between the instrument shelter and station 9.

11.—At the east edge of the woods, in tall grass. This station was selected after the interference with the atmometer at Station 7.

Table F gives the data obtained from this series of observations. Graphs in figure 2 illustrate the relations between the amounts of evaporation at the different stations.

Beginning at a rather high level during the first week of observation, evaporation reached a maximum during the week ending July 18. This was followed by a rapid fall and a minimum during the week ending September 5. From this point there was a gradual rise until October 24, when a second maximum occurred. The first maximum was due to high temperature and lack of rainfall, while the minimum following was due to a heavy rainfall, without, however, much diminution of temperature. The second maximum was due to a lack of rainfall and to greater air movement and insolation due to the falling of the leaves. The instrument at Station 1 was left until it was broken by freezing, during the week ending November 14. Atmometers were again replaced at four of the stations when danger of freezing was past in the spring. A high maximum was reached during the week of May 8, which was followed by a sharp decline due principally to the greater development of the foliage.

Although there are a few minor exceptions, the data show a constant gradient in the evaporating power of air, from the ground level upward. This is what would be expected in view of the gradient in humidity, temperature and light. Wind movement, also, probably increases upward, although no instrumental measurements of this factor were made.

It will be seen that, although the difference between the evaporation at Station 1 and half a meter higher on the trunk of the small tree (Station 3) was very small, there were only six weeks during the entire season when a difference was not recorded. The difference between the north and south sides of the trees was negligible except during the latter part of October, when the amount evaporated was about 0.9 cc per day less on the north side. This difference was evidently due to the greater exposure to sunlight of the instrument on the southern side. The differences between the 1 m level and the 1.5 m level were greatest during the weeks of August 1, August 15, and September 5. These were weeks of comparatively great rainfall, when the lower air strata were thoroughly saturated with moisture, due to the greater amount of water in the soil.

The evaporation from atmometer No. 5, suspended from a maple branch 1.5 m above No. 1, and 1 m above the instruments just discussed, followed that obtained from the latter very closely, falling below in two instances, the weeks of August 1 and April 24.

The evaporation from atmometer No. 9, at 10 m followed, in a general way, that at the stations previously discussed. It was, in almost every instance, greater than at the other stations, the exceptions being the weeks of September 26 and October 10. These exceptions were slight, and can be explained by the fact that the gradient became less marked at this time on account of the fall of the leaves, and that slight variations in any direction might be expected due to the later fall of leaves from some trees than from others.

The evaporation from atmometer No. 8, at 6 m above the surface of the ground fell between that from Nos. 5 and 9, except for the week of September 5, when it was higher than either. For the weeks of September 26 and October 3 it was practically the same as No. 9. The rise in the last three weeks is probably explainable by earlier fall of the leaves of the elm tree and the greater exposure to the west wind.

Atmometer No. 10, at the upper margin of the forest crown showed the greatest evaporation of all. The drop in the evaporation from this instrument and from No. 8 during the week of September 5 was partially due to the accumulation of air in the porous cups. The readings are given, however, as they were obtained.

Atmometer No. 2, near the ground varied almost exactly with No. 1, with an average difference of 3.2 cc per day.

The evaporation in the hollow stump (atmometer Number 6) followed that from atmometer No. 2 except, apparently, for the weeks ending July 11 and 18. This discrepancy was probably due to an error in filling the bottle on the former date. It was located in very poor light, and an error of that kind was very easy to make.

The evaporation from the forest edge stations is not illustrated in the figure. That at the west forest edge, in short grass, was very much higher than elsewhere, with the exception of the tree tops. At the east forest edge in long grass, the evaporation was about the same as at 6 m in the woods.

The comparative evaporation at the various stations was computed as the average daily rate between July 1 and October 10. In the case of Stations 1 to 6 inclusive, the calculations were made directly. In the case of the others, where observations did not cover the entire period, the result was obtained by comparing the evaporations recorded with those recorded at Station 1 for the same dates, and assuming that the relative ratios would hold for the entire season. The values thus obtained were as follows:

Station	1	2	3	4	5	6	7	8	9	10	11
Mean weekly evaporation cc.	9.16	5 96	9.90	9.63	10.4	4.14	14 03	12.30	12.31	15.71	11.91

Similar calculations were not made from the spring series of observations, but similar relations were indicated.

The gradient of the evaporating power of air in the forest is very striking, stations less than a meter apart, vertically, showing definite and constant differences. This is particularly true at the lower levels. At the higher levels the gradient still persists but is subject to irregularities due to the greater exposure and lack of uniform conditions. The steepest gradient, also, is near the ground. An organism passing from the air stratum just above the soil to the top of a tree would enter a region of three times the average evaporating power, and would encounter a still greater diurnal variation in evaporating power. Evaporating power is more than doubled in passing from the herb stratum to the upper shrub stratum. It is probable that conditions under fallen leaves and debris are somewhat approximated by those in the hollow stump. An animal traveling from the interior of such a hollow stump to its top would encounter double the evaporating power. Such considerations serve to point out the extreme importance of this environmental factor and to emphasize the steepness of the gradient in that region of the forest in which stratification of animals is most complete.

3. *Temperature.*—Temperature data were obtained mainly by the use of recording thermographs. During the greater part of the period of study three such instruments were in use, although minor accidents at times prevented the proper functioning of one or the other for short periods of time, and one failed almost entirely during the winter period of low temperature.

The sensitive element of the instrument recording soil temperatures was placed with its upper surface 10 cm below the surface of the soil, which was, in turn, covered by the usual carpet of leaves and other debris, making, perhaps, in all a layer 15 cm in thickness above the thermometer bulb. Of the air temperature instruments, the sensitive element of one was exposed on the north side of the instrument shelter previously mentioned, within which the mechanical parts of the two instruments were housed. The bulb of the instrument was protected from the direct rays of the sun and from falling water, but was exposed to the free circulation of the air.

The third instrument was of the self-contained type, and was suspended about 10 m above the surface of the ground with the hygrograph previously mentioned. The clock mechanism of this thermograph did not work well during the colder part of the year, and only partial readings were obtained after December 5. Figures obtained from this instrument after this date are not included in the tabulations or graphs.

A standard Weather Bureau type maximum and minimum thermometer set was also installed in the instrument shelter, with the bulbs exposed approximately as in the case of the recording instrument. This was used in checking the thermographs. The thermograph in the tree could, of course, be checked with the mercury thermometers only when brought to the ground, which was done each week in order to change the record sheets.

The weekly record sheets were changed on Monday morning as in the case of the hygrographs. The data obtained from the thermograph records were treated in substantially the same way as those of the hygrographs, and the results are similarly tabulated and illustrated.

The summaries of the air temperature data at 60 cm and at 10 m are given in Tables C and D (Appendix), and the mean temperatures and mean variations at 60 cm are illustrated graphically in figure 1, C and E. The highest temperatures occurred during the first weeks of the period of observation, and there was a gradual decline until the week of January 30. Marked variations from the general downward trend were noted during the weeks of September 5, October 31, November 21, and January 9. The most rapid declines in temperature were between October 31 and November 14 and between January 9 and January 23. After January 30 the trend was again upward, with reversals occurring during the weeks of February 20, March 6, April 24, and May 22. The variation, from week to week, of the mean range was almost as great as that of the mean temperature. The excessive temperatures of the first weeks were accompanied by high variability but thereafter, until the end of October, and during the spring, the higher temperatures were accompanied by a smaller range. During the winter, however, high temperatures were associated with a great mean daily range. In other words, the high means of mid-summer were occasioned by a lack of the usual low nightly minimum. The chief factor in producing a low mean in the winter was the absence of a high mid-day rise. The relation existing between the maxima and the "base means" is accordingly of importance.

With a few exceptions the weekly mean temperatures at 10 m were higher than those recorded near the ground. Exceptions to this rule were recorded for the entire month of October and a portion of November. Observations at the German forest meteorological stations showed, for deciduous forests, a reversal of the ordinary forest temperature gradient coinciding with the fall of the leaves. Thus, during the leafless season, the forest agreed with the open country in exhibiting a decrease of temperature upward. If the observations at the higher level had been continued throughout the winter it is probable that this reversal would have become more evident. The mean difference in temperature at the two stations during the period of complete records at both levels was 0.36° C., which would correspond to a difference of 1.94° F. per hundred feet, or approximately the figures (2° F) obtained by the German investigators, according to Harrington (1893).

The data of Lorenz-Liburnau (1890) show an average difference of temperature between ground level and 11 m in beech woods, according to numerous scattered observations, as follows:

Time	Temperature at 0.0 m. Minus Temperature at 11 m.
Early morning (2:55-5:25 a.m.)	−0.1°C
Forenoon (6:15-11:00 a.m.)	0.35°
Mid-day (11:00 a.m.-3:00 p.m.)	1.07°
Late afternoon (3:00-6:40 p.m.)	1.09°
Evening (5:50-9:55 p.m.)	0.44°

These observations covered the period between May 23 and October 28, near Vienna. While it was not practicable to calculate the differences for the various hours of the day throughout the period of observations, this was done for a week of average days without disturbing influences. The hygrothermograph records for this week (ending Sept. 12) are reproduced as Fig. 7. A compilation of the numerical data for each two-hour period of the day gives the following results:

Week Ending Sept. 12, 1921—See Figure 6

Time	Mean Temp. at 10.0 m.	Mean Temp. at 0.6 m.	Difference
6-8 a.m.	16.28°C	16.11°C	0.17°C
8-10	19.39	18.17	1.22
10-12 m.	22.06	20.78	1.28
12-2 p.m.	23.33	22.00	1.33
2-4	23.11	22.17	0.94
4-6	21.89	21.67	0.22
6-8	20.61	20.00	0.61
8-10	18.72	19.11	−0.39
10-12 n.	18.11	17.94	0.17
12-2 a.m.	17.17	17.44	−0.27
2-4	16.44	16.61	−0.17
4-6	15.39	16.33	−0.94
		Average Difference	0.34°C

The average difference for this week is of the same order of magnitude as the average for the period of complete records as previously given. The variations in the vertical temperature gradient during the nycthemeral cycle also seem analogous to its variations during the annual cycle, in that a reversal takes place during the cooler periods in each case.

As the temperature at the higher station was greater during the warmer hours, and lower during the early morning, it follows that the mean variability of temperature was greater at this level. This was true during the summer and early autumn months. During the winter the mean variability was greater at the lower level, due, probably, to the greater effect of the sun on the temperature near the ground after the disappearance of the protective leaf-canopy.

Soil temperature was much more uniform than air temperature, and that within the forest, according to numerous observers, is much more uniform than that in the open at an equal depth. This is due, largely, to the lack of insolation and to the protective blanket of leaves and other ground litter. During the months when the deciduous trees were in leaf the maxima came at about 4 p.m. and the minima about 8 a.m., although sometimes delayed until 10 a.m. The variations became greater as the leaves fell, then decreased again as the ground froze, remaining very slight throughout the winter. A sudden rise, accompanied by daily variations of as much as 4° C. occurred during the week of March 13. This represented the temperature changes accompanying the thawing of the soil moisture during a series of early spring rains. Until the middle of May variability of soil temperature remained high.

Soil temperature data are given in Table E (Appendix) and illustrated in Figure 2. Although the mean soil temperature showed variations in the main closely correlated with those of the air near the surface of the soil, this mean, during the summer was about 5° below the air mean. Beginning with the week of November 14, a lag became apparent and the magnitude of the changes from week to week became decidedly less in the soil. During this period the mean temperature of the soil averaged higher than that of the air, thus reversing the condition found in the warmer season. The spring reversal occurred during the week of March 13, a period of heavy rain.

In summer the temperature increases from the soil upward to the forest crown; in winter the highest mean temperatures are in the soil and temperature decreases upward. In summer the temperature is most variable in the forest crown and least so in the soil; in winter the soil is still the most stable, but the greatest variation is found in the air stratum just above the soil.

4. *Light*—It was not possible, on account of lack of time and the unsuitability of apparatus for continuous observation, to conduct extensive observations on the quality and quantity of light available for organisms at various levels of the woods, and at various times. However, a short series of readings was made with the Macbeth Illuminometer. For details see Table G, Appendix. These readings were taken in the period between noon of August 29 and noon of August 30, while supplementary readings were taken on July 30 and August 22. Both of the days last mentioned, however, were unfavorable for comparative observations on account of partial cloudiness. On August 29 and on the day following, until about noon, the sky was clear, although somewhat hazy, and clouds did not begin to interfere with the readings until after noon.

Observations were taken at the ground level, under the shelter of a leaf mosaic and at the height of 1.25 m in a group of tall maples with fairly dense foliage, and in a sunny glade at the western edge of the woods. The test plate was horizontal in position in every case, and an amber filter was used to equalize the color values of the standard lamp and daylight.

Additional readings were taken at each station with the use of color screens, but as it has been impossible to obtain a spectrophotometric analysis of these screens in comparison with a similar analysis of the light emitted by the standard lamp, the data thus obtained are omitted here. The results indicated, however, a variation through the day in the relative constitution of the light as well as a difference in the quality of the light at different levels in the forest and in the open glade. As observations could not be taken at any two places at exactly the same time, any evidence based upon small differences must remain inconclusive.

As to intensity of light the results were much more definite. The light at 1.25 m in the shrub stratum but once during the day reached one per cent of the intensity of full noon sunlight. The maximum reading at this level, at noon, being 1170 meter-candles, while that in an open glade in the forest at the same height, in full sunlight, was 96650 meter-candles. At the ground level, under cover of herbage the highest reading was about 0.35 per cent of full sunlight, or 378 meter-candles. Both of the forest readings were taken in the shade. Throughout the day, flecks and small patches of sunlight find their way through the leafy canopy. Their intensity is difficult to measure, but the smaller flecks probably approximate one-half of full sunlight intensity, and larger areas have their intensity but little diminished. The difference between light at the lower levels in the forest and that at corresponding levels outside is very large, and the ratio of light intensity between the ground stratum and the shrub stratum may be as high as one to three or four. This factor has been little considered in connection with the study of the distribution of animals, but it deserves thorough consideration now that instruments for its measurement are being developed.

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PLATE I

EXPLANATION OF PLATE I

Animal Population Data.

Total population, and population of the shrub, herb, leaf, and ground strata of an area of four square feet in the forest.

A—Total population.
B—Population of the herb stratum.
C—Population of the shrub stratum.
D—Population of the leaf stratum.
E—Population of the soil stratum.

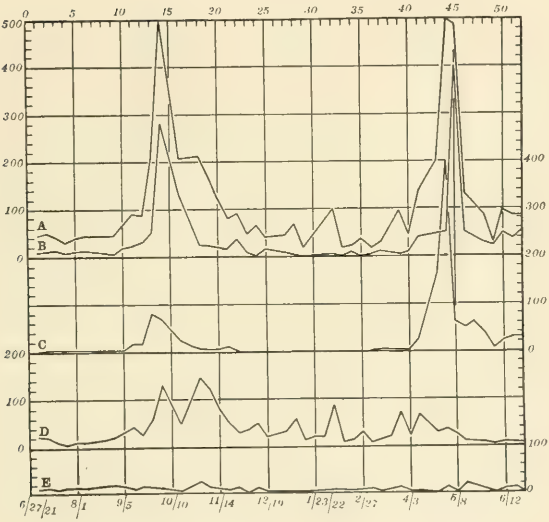

PLATE I

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

PLATE II

EXPLANATION OF PLATE II

Temperature, Humidity and Evaporation Data.

Time is represented horizontally, each smaller division corresponding to one week, beginning with the week ending July 4, 1921.

A—Mean relative humidity at Station A, 0.6 M. above ground surface.
B—Mean daily variation in relative humidity at Station A.
C—Mean Temperature at Station A, 0.6 M. above ground surface.
D—Mean soil temperature, Station A, 0.1 M. below ground surface.
E—Mean daily variation in air temperature, Station A.
F—Mean daily variation in soil temperature, Station A.
G—Average daily evaporation from a standard porous cup atmometer at Station 3 (See page 57).
H—Average daily evaporation at Station 1.
I—Average daily evaporation at Station 2.
J—Average daily evaporation at Station 9.
K—Average daily evaporation at Station 5.
L—Average daily evaporation at Station 6.
M—Mean daily evaporation for the entire season from each station.

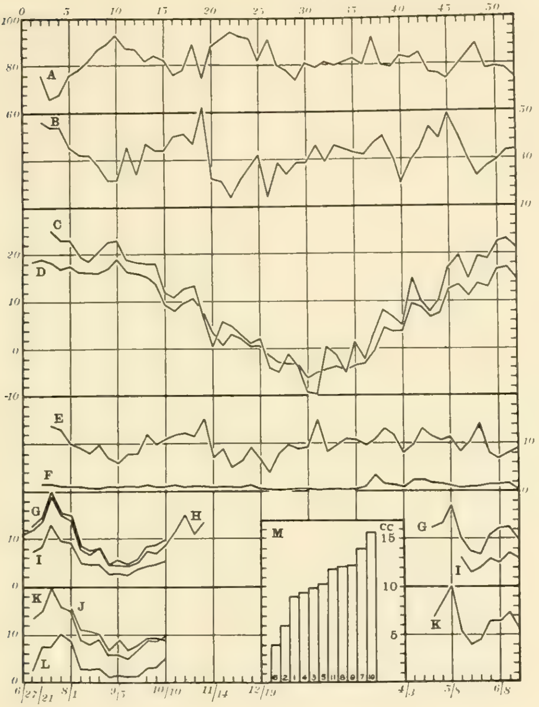

PLATE II

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

PLATE III

EXPLANATION OF PLATE III

Seasonal Occurrence of Insects (Coleoptera).

Time is represented as in Figure 1. The vertical scale is relative, only, and is not the same for all species.

The solid line represents the relative numbers found in the leaf-soil strata, the broken line the herb stratum, and the dotted line the shrub stratum.

A—Diabrotica vittata Herbst.
B—Notoxus monodon Fab.
C—Phalacrus politus Melsh.
D—Phytonomus nigrirostris Fab.
E—Telephanus velox Hald.
F—Glyptina spuria Lec.
G—Epitrix brevis Schw.
H—Epitrix fuscula Crot.
I—Chaetocnema confinis Crot.
J—Phyllotreta sinuata Steph.

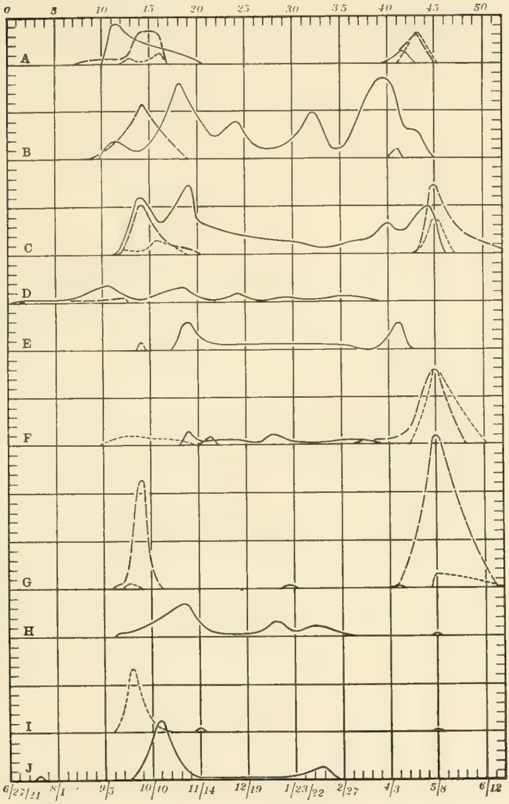

PLATE III

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

PLATE IV

EXPLANATION OF PLATE IV

Seasonal Occurrence of Insects (Coleoptera and Hemiptera) and Spiders.

In Graph K the different strata are indicated as in Figure 4. In Graphs P, Q and T, the heavy lines represent adult spiders and the light lines young spiders. In Graphs L, M, N, O, R, and S the total population only is shown.

K—Longitarsus melanurus Melsh.
L—Corythucha aesculi O & D.
M—Corimelaena pulicaria (Germ.).
N—Lygus pratensis (L.).
O—Blissus leucopteris (Say).
P—Dendryphantes aestivalis Emer.
Q—Uloborus americanus Walck.
R—Anyphaena rubra Emer.
S—Dictyna sp?
T—Xysticus elegans Keys.

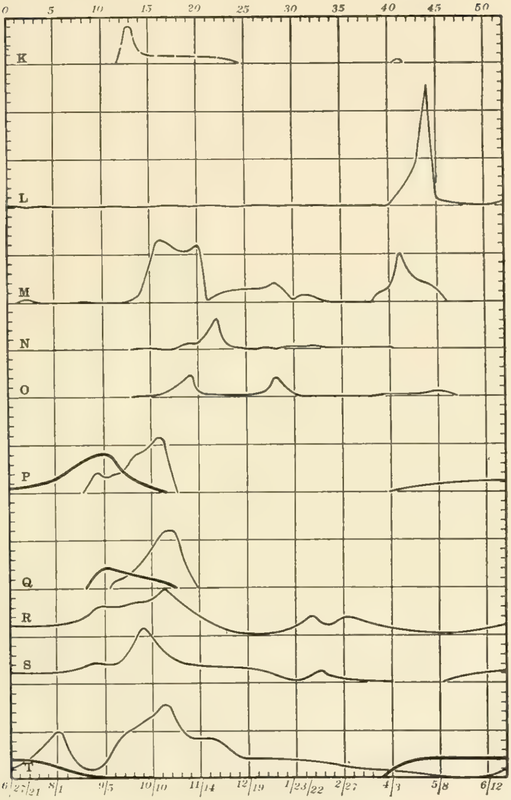

PLATE IV

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

PLATE V

EXPLANATION OF PLATE V

Seasonal Occurrence of Spiders and Insects (Cicadellidae).

For explanation see Figures 4 and 5.

U—Linyphia phrygiana Koch.
V—Acrosoma rugosa Hentz.
W—Epeira gibberosa Hentz.
X—Tetragnatha sp?
Y—Empoasca viridescens Walsh.
Z—Total Cicadellidae and Erythroneura obliqua Say.

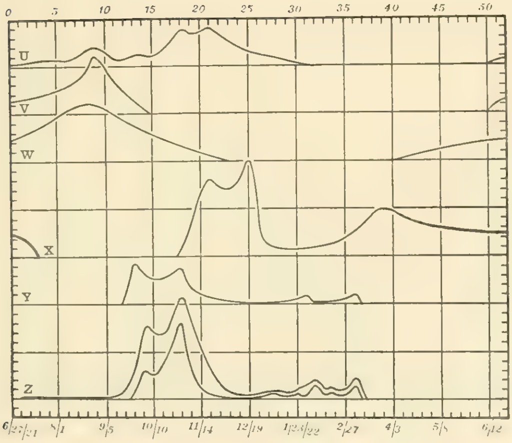

PLATE V

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

PLATE VI

EXPLANATION OF PLATE VI

Temperature and Humidity Data.

Mean temperature and mean relative humidity of an ideal day (computed from the two-hour means for the week ending September 13, 1921) at the 0.6 m. and 10 m. levels in the forest.

A—Humidity.
B—Temperature.

The upper curve of A and the lower curve of B represent conditions at the 0.6 m. level.

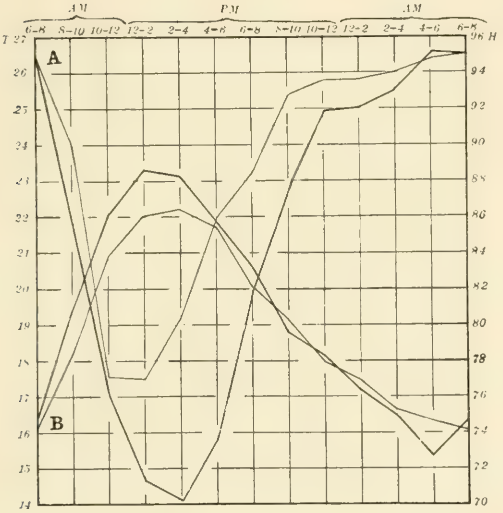

PLATE VI

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

PLATE VII

EXPLANATION OF PLATE VII

Temperature and Humidity Data

Hygrothermograph records for the week ending September 12, 1921, at the 0.6 m. and 10 m. levels.

The lower curve in each chart is the temperature record. The temperature range of the chart is indicated. 100% humidity is at 38° and 50% humidity at 10° on the chart.

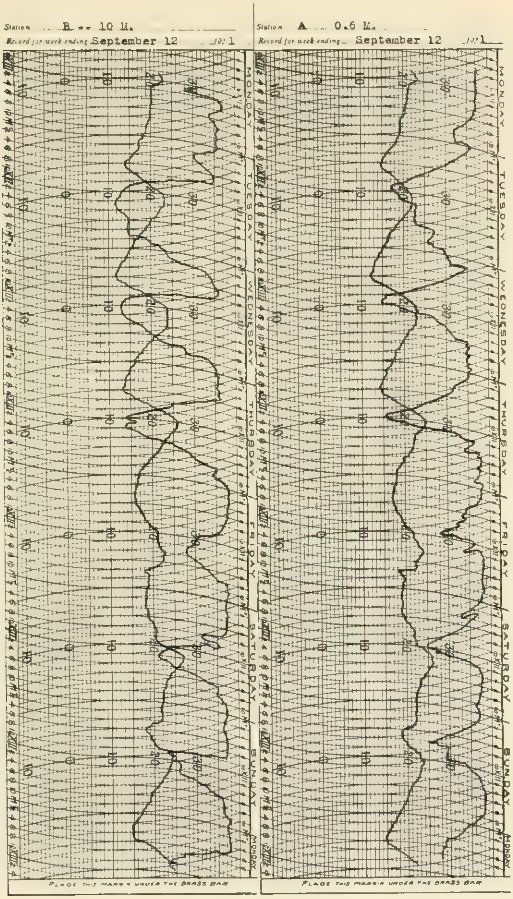

PLATE VII

ANIMAL ECOLOGY OF AN ILLINOIS ELM-MAPLE FOREST

WEESE

TABLE A

Relative Humidity Data Station A, 0.6 m. above ground surface

Week ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean R. H.	Base Mean	Total Range	Mean Range	Mean Range Below Base
July 11	2	100	40	93.7	48.0	74.7	87.1	60	45.7	39.1
July 18	3	94	35	84.1	40.4	66.1	76.5	59	43.7	36.1
July 25	4	95	35	86.1	41.7	68.6	79.4	60	44.4	37.7
Aug. 1	5	94	42	88.1	53.5	75.8	83.3	52	34.6	29.8
Aug. 8	6	95	42	90.0	58.5	77.6	85.2	53	31.5	25.7
Aug. 15	7	96	42	94.4	62.8	82.4	91.2	55	31.6	28.4
Aug. 29	8	100	64	98.1	70.5	86.8	92.2	36	27.6	21.7
Sept. 5	9	100	59	99.9	78.3	89.2	96.5	31	21.6	18.2
Sept. 12	10	100	61	99.8	78.0	93.2	97.7	39	21.8	19.7
Sept. 19	11	100	52	97.5	62.5	87.5	91.2	48	35.0	28.7
Sept. 26	12	99	58	95.7	71.5	87.2	91.2	41	24.2	19.7
Sept. 26	13	100	47	95.1	58.0	82.3	90.2	53	37.1	32.3
Oct. 3	14	100	43	96.0	62.1	84.1	91.9	57	33.9	28.9
Oct. 10	15	100	55	95.9	63.3	81.8	90.0	45	33.6	27.6
Oct. 17	16	100	46	96.0	56.0	76.1	90.8	54	40.0	34.8
Oct. 24	17	100	49	95.5	54.4	77.9	87.8	51	41.1	33.4
Oct. 31	18	100	41	100.0	63.0	88.6	96.4	59	37.0	34.4
Nov. 7	19	100	39	96.0	44.4	75.5	91.3	61	51.6	46.9
Nov. 14	20	100	59	95.5	73.3	88.1	92.5	41	22.2	19.2
Nov. 21	21	100	52	98.1	77.0	91.3	95.3	48	21.1	18.3
Nov. 28	22	100	60	99.3	85.3	94.5	97.6	40	14.0	12.3
Dec. 5	23	100	68	98.7	77.7	92.1	97.4	32	21.7	20.4
Dec. 12	24	100	63	99.4	72.7	90.8	96.4	37	26.7	23.7
Dec. 19	25	100	50	93.0	60.3	82.5	87.7	50	32.7	27.4
Dec. 26	26	100	57	95.3	81.9	91.4	93.5	43	13.5	11.7
Jan. 2	27	95	38	86.3	57.7	79.3	82.0	57	28.6	24.3
Jan. 9	28	100	47	86.0	60.5	78.5	53.0	25	23.3	23.3
Jan. 16	29	100	48	87.4	58.8	74.0	81.3	52	28.6	22.5
Jan. 23	30	100	50	91.7	63.1	81.2	88.4	50	28.6	25.3
Jan. 30	31	98	51	91.8	56.7	79.3	85.7	47	35.1	29.0
Feb. 6	32	100	43	92.4	63.3	81.3	86.7	57	29.1	23.4
Feb. 13	33	100	48	92.3	55.8	80.3	86.2	52	36.5	30.4
Feb. 20	34	98	44					54		
Feb. 27	35	100	42	97.0	64.0	82.9	91.8	58	33.0	27.8
Mar. 6	36	100	58	95.0	63.1	80.0	88.7	42	31.9	25.6
Mar. 13	37	100	47	97.0	60.0	91.6	93.6	53	37.0	23.6
Mar. 20	38	100	31	94.0	54.9	80.2	89.8	69	39.1	34.9
Mar. 27	39	100	46	94.9	63.9	79.2	89.0	54	31.0	25.1
Apr. 3	40	96	47	92.1	72.6	83.9	89.4	49	19.5	16.8
Apr. 10	41	99	50	97.7	68.4	82.9	89.5	49	29.3	21.1
Apr. 17	42	100	44	98.4	64.1	84.8	94.5	56	34.3	20.4
Apr. 24	43	97	43	94.3	50.7	76.6	88.6	54	43.6	37.9

Week ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean R. H.	Base Mean	Total Range	Mean Range	Mean Range below Base
May 1	44	100	31	93.6	53.9	76.1	88.8	69	39.7	34.9
May 8	45	100	40	94.3	46.4	73.8	87.8	60	48.9	41.4
May 15	46	100	45	94.4	53.6	79.2	88.3	55	40.8	34.7
May 22	47	100	55	97.3	66.9	84.8	92.4	45	30.4	25.5
May 29	48	100	60	95.6	72.0	88.6	93.2	40	23.6	21.2
June 5	49	95	57	88.7	61.6	77.9	83.6	38	27.1	22.0
June 12	50	100	54	93.1	64.0	78.9	87.5	46	29.1	23.5
June 19	51	100	50	93.6	60.1	78.0	85.3	50	33.5	25.2
June 26	52	92	47	85.6	51.3	69.6	75.0	45	34.3	23.7

TABLE B

Relative Humidity Data Station B, 10 m. above ground surface

Week ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean R. H.	Base Mean	Total Range	Mean Range	Mean Range Below Base
Aug. 22	8	94	58	93.1	59.8	79.0	88.7	36	33.3	28.9
Aug. 29	9	100	62	96.0	68.8	84.9	92.8	38	27.5	24.0
Sept. 5	10	100	57	100.0	69.5	90.2	95.4	43	30.5	25.0
Sept. 12	11	97	47	61.0	83.3	91.6	50.0	36	36.6	30.6
Sept. 19	12	90	35	88.0	55.3	77.5	86.2	55	32.7	30.9
Sept. 26	13	90	34	89.6	47.3	73.4	84.6	56	41.8	36.8
Oct. 3	14	97	38	95.4	56.0	81.0	92.2	59	38.4	36.2
Oct. 10	15	100	45	98.9	54.5	79.1	88.4	55.	44.3	33.9
Oct. 17	16	100	42	95.1	52.3	75.1	86.3	58	42.8	43.1
Oct. 24	17	100	40	92.0	50.0	73.6	92.2	60	42.0	32.2
Oct. 31	18	100	41	100.0	64.4	88.6	96.9	59	35.6	31.5
Nov. 7	19	97	36	93.4	46.4	73.4	87.3	61	47.0	41.0
Nov. 14	20	100	62	98.0	72.0	86.9	92.4	38	26.0	20.4
Nov. 21	21	100	50	86.3	80.1	91.8	92.6	50	16.2	12.5
Nov. 28	22									
Dec. 5	23	100	60	96.0	70.0	85.7	92.0	40	26.0	22.0
Dec. 12	24	100	47	99.1	63.4	86.0	92.7	53	35.7	29.3
Dec. 19	25	100	34	94.3	58.0	81.9	87.4	66	36.2	29.4
Dec. 26	26	100	66	96.5	82.3	93.6	93.6	34	14.2	11.3
Jan. 2	27	100	23	96.0	52.5	77.5	80.8	77	43.5	28.3
Jan. 9	28	100	49	84.1	56.0	74.2	78.2	51	28.1	22.2
Jan. 16	29	100	35	86.7	49.8	68.5	76.7	65	36.9	26.9
Jan. 23	30	100	38	92.3	57.7	79.9	85.8	62	34.6	28.1
Jan. 30	31	96	46	91.5	51.0	75.5	82.3	50	40.5	31.3
Feb. 6	32	100	48	50.7	76.5	83.0	52.0	52	42.7	32.3
Feb. 13	33	100	42	94.3	50.4	77.5	84.7	58	43.9	34.3

TABLE C

Temperature Data Station A, 0.6 m. above ground surface

Week ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean Temp.	Base Mean	Total Range	Mean Range	Mean Range Above Base
July 4	1	32.2	22.2					10.0		
July 11	2	33.3	23.3					10.0		
July 18	3	34.2	18.9	33.2	19.5	25.5	21.3	15.3	13.7	11.9
July 25	4	34.4	13.9	30.9	17.8	23.2	19.9	20.6	13.1	10.9
Aug. 1	5	33.3	14.4	29.4	19.4	23.7	21.5	19.0	10.0	7.9
Aug. 8	6	27.8	14.4	25.3	15.9	19.7	16.9	13.3	9.4	8.4
Aug. 15	7	27.5	11.7	23.7	15.5	18.4	17.2	15.8	8.2	6.6
Aug. 22	8	30.0	14.7	25.6	15.9	20.4	18.0	15.3	9.7	7.6
Aug. 29	9	33.3	15.0	27.7	20.3	21.6	20.9	18.3	7.4	6.8
Sept. 5	10	30.0	19.4	26.4	20.3	23.1	21.7	10.6	6.1	4.8
Sept. 12	11	29.2	11.7	23.2	15.1	18.9	17.3	17.5	8.1	5.8
Sept. 19	12	28.3	9.4	22.9	14.8	18.4	17.0	18.9	8.2	5.9
Sept. 26	13	27.5	8.1	27.0	15.1	18.1	15.7	19.4	11.9	11.3
Oct. 3	14	29.4	9.2	23.1	13.4	18.1	15.8	20.3	9.7	7.3
Oct. 10	15	23.3	2.2	17.6	6.5	12.0	8.7	21.1	11.1	8.8
Oct. 17	16	25.6	2.2	17.4	5.5	10.9	7.7	23.3	11.9	9.7
Oct. 24	17	25.6	4.4	20.2	7.8	12.8	9.9	21.1	12.4	10.3
Oct. 31	18	22.8	7.2	20.6	8.9	13.6	10.8	15.6	11.7	9.8
Nov. 7	19	18.3	− 1.7	14.8	− 0.5	6.7	2.9	20.0	15.3	11.9
Nov. 14	20	13.9	− 7.8	4.4	− 2.9	0.7	− 0.5	21.7	7.4	4.9
Nov. 21	21	17.8	− 5.0	10.5	1.4	5.8	3.8	22.8	9.1	6.7
Nov. 28	22	10.6	− 3.3	7.6	2.1	4.9	4.0	13.9	5.5	3.6
Dec. 5	23	14.4	− 3.3	7.0	0.6	3.3	2.3	17.8	6.4	4.7
Dec. 12	24	11.7	− 6.7	6.7	− 2.6	1.3	− 0.8	18.3	9.3	7.4
Dec. 19	25	12.8	−10.0	6.4	− 0.6	2.0	1.3	22.8	7.1	5.1
Dec. 26	26	8.6	− 9.4	− 1.9	− 5.8	− 4.0	− 4.8	18.1	3.8	2.9
Jan. 2	27	3.9	−12.2	− 0.7	− 8.8	− 4.9	− 6.7	16.1	8.1	6.0
Jan. 9	28	12.8	−10.0	4.5	− 5.4	− 0.8	− 4.1	22.8	9.9	8.6
Jan. 16	29	8.3	−15.8	− 1.6	−10.6	− 3.7	− 7.4	24.2	9.0	5.8
Jan. 23	30	3.1	−23.3	− 3.6	−13.0	− 9.3	−10.0	26.4	9.4	6.3
Jan. 30	31	1.7	−19.4	− 0.9	−15.7	− 9.8	−14.0	21.1	14.8	13.1
Feb. 6	32	8.6	− 7.5	5.2	− 3.3	0.3	− 1.2	16.1	8.5	6.4
Feb. 13	33	14.2	−15.0	4.7	− 4.9	− 1.4	− 3.3	29.2	9.6	7.9
Feb. 20	34	10.6	−17.8	1.2	− 9.8	− 4.9	− 7.8	28.4	11.0	9.0
Feb. 27	35	21.7	− 7.2	8.4	− 2.2	1.7	− 0.6	28.9	10.6	9.0
Mar. 6	36	11.5	− 9.2	3.7	− 6.1	− 2.1	− 4.9	20.7	9.8	8.6
Mar. 13	37	14.7	− 4.2	10.3	− 0.6	3.6	0.7	18.9	10.9	9.6
Mar. 20	38	22.8	− 0.3	16.4	3.2	8.3	5.3	23.1	13.2	11.1
Mar. 27	39	22.5	− 3.3	13.5	2.0	7.1	3.8	25.8	11.5	9.7
Apr. 3	40	15.0	− 1.1	10.6	2.7	5.7	3.8	16.1	7.9	6.8
Apr. 10	41	25.3	8.3	20.3	10.6	14.7	11.9	17.0	9.7	8.4
Apr. 17	42	25.0	0.0	17.6	4.6	10.1	7.2	25.0	13.0	10.4

Week ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean Temp.	Base Mean	Total Range	Mean Range	Mean Range Above Base
Apr. 24	43	19.2	0.0	15.1	3.5	7.8	4.9	19.2	11.6	10.2
May 1	44	23.6	1.1	16.8	6.0	10.5	7.1	22.5	10.8	9.7
May 8	45	25.0	10.6	23.7	12.3	17.3	13.6	14.4	11.4	10.1
May 15	46	28.1	11.1	24.9	16.7	19.9	17.7	17.0	8.2	7.2
May 22	47	21.7	11.4	19.3	13.0	14.7	13.0	16.3	10.3	6.3
May 29	48	25.8	12.2	23.2	16.7	19.5	17.9	16.5	13.6	5.3
June 5	49	23.9	12.8	22.8	15.0	18.8	16.4	11.1	7.8	6.4
June 12	50	30.3	16.7	26.1	19.3	22.6	20.6	13.6	6.8	5.5
June 19	51	31.1	15.0	27.1	18.9	23.0	21.1	16.1	8.2	6.0
June 26	52	32.2	13.9	26.1	17.2	21.6	19.1	18.3	9.9	7.0

TABLE D

Temperature Data Station B, 10 m. above ground surface

Week ending	No. Week	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Temp. Mean	Base Mean	Total Range	Mean Range	Mean Range Above Base
Aug. 1	5	33.3	13.9	30.5	20.9	24.6	21.4	19.4	9.6	9.1
Aug. 8	6	26.7	14.4	25.3	15.4	19.9	16.9	12.2	9.9	8.4
Aug. 15	7	28.3	9.4	24.3	15.6	19.6	17.1	18.9	8.7	7.2
Aug. 22	8	31.1	11.1	25.7	16.2	20.8	18.0	20.0	9.6	7.7
Aug. 29	9	30.6	15.0	23.1	19.7	23.4	21.0	15.6	8.4	7.1
Sept. 5	10	30.6	18.9	27.1	19.6	22.9	21.1	11.7	7.4	5.9
Sept. 12	11	26.7	11.7	24.8	14.8	19.4	17.1	15.0	10.1	7.7
Sept. 19	12	27.2	8.9	32.1	15.0	18.4	16.6	18.3	8.1	6.5
Sept. 26	13	31.7	7.2	25.1	13.9	19.2	17.1	24.4	11.2	7.9
Oct. 3	14	32.2	9.4	23.9	13.7	17.7	15.0	22.8	10.2	8.9
Oct. 10	15	29.4	0.6	17.8	5.8	11.8	8.7	28.9	12.1	9.1
Oct. 17	16	26.7	− 0.6	17.3	4.9	10.1	7.2	27.2	12.4	10.1
Oct. 24	17	27.8	2.2	19.1	6.8	12.1	9.0	25.6	12.2	10.1
Oct. 31	18	26.7	0.0	19.3	9.3	13.3	11.1	26.7	10.0	8.2
Nov. 7	19	18.3	− 1.7	14.2	3.3	8.2	4.8	20.0	10.9	9.4
Nov. 14	20	16.7	− 3.9	6.6	− 0.5	2.4	1.6	20.6	7.1	5.0
Nov. 21	21	18.9	− 3.9	9.7	3.4	6.5	4.9	22.8	6.3	4.8
Nov. 28	22	12.8	− 2.2	6.7	1.1	4.3	2.8	26.1	5.6	3.9
Dec. 5	23	13.9	− 2.2	7.8	2.2	4.3	3.5	16.1	5.6	4.3

TABLE E

Temperature Data—Soil Temperature 0.1 m. below surface of soil

Week Ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean Temp.	Extreme Range	Mean Daily Range
July 4	1	20.0	18.0	19.7	18.1	18.7	2.0	1.6
July 11	2	20.2	18.3	19.9	18.3	19.0	1.9	1.6
July 18	3	20.0	18.1	19.3	18.1	18.3	1.9	1.2
July 25	4	20.0	15.6	18.0	16.8	17.4	4.4	1.2
Aug. 1	5	20.3	16.1	19.2	17.9	17.8	4.2	1.2
Aug. 8	6	18.3	15.0	16.9	16.1	16.5	3.3	0.8
Aug. 15	7	18.3	14.7	16.6	15.8	16.3	3.6	0.8
Aug. 22	8	18.3	14.4	17.0	15.6	16.2	3.9	1.4
Aug. 29	9	19.2	14.4	17.4	16.3	16.9	4.7	1.1
Sept. 5	10	19.4	17.8	19.5	18.2	18.8	1.7	1.3
Sept. 12	11	18.3	15.3	17.2	16.1	16.7	3.1	1.1
Sept. 19	12	17.2	13.9	16.7	15.0	16.1	3.3	1.7
Sept. 26	13	17.5	12.2	15.9	14.6	15.5	5.3	1.3
Oct. 3	14	18.1	11.1	15.1	13.8	13.9	6.9	1.3
Oct. 10	15	11.7	8.6	10.4	8.8	9.5	3.1	1.6
Oct. 17	16	10.8	6.1	8.6	7.6	8.1	4.7	0.9
Oct. 24	17	12.5	7.2	10.3	9.2	5.3	5.3	1.2
Oct. 31	18	12.2	9.4	11.3	10.2	10.9	2.8	1.2
Nov. 7	19	9.7	5.6	7.8	6.2	7.1	4.2	1.6
Nov. 14	20	6.7	0.0	3.8	2.5	3.2	6.7	1.3
Nov. 21	21	1.4	0.0	1.4	0.0	0.8	1.4	1.4
Nov. 28	22	4.4	1.7	4.0	2.9	3.3	2.8	1.1
Dec. 5	23	6.1	0.6	3.4	2.3	2.9	5.6	1.2
Dec. 12	24							
Dec. 19	25	4.7	− 2.8	− 1.4	− 1.9	0.8	6.1	1.5
Dec. 26	26	0.8	− 1.4	− 1.4	− 1.9	− 1.6	3.6	0.5
Jan. 2	27	− 2.8	− 3.9	− 2.8	− 3.1	− 2.9	1.1	9.3
Jan. 9	28	− 2.8	− 3.3	− 3.1	− 3.3	− 3.2	0.6	0.2
Jan. 16	29	− 2.8	− 4.7	− 3.4	− 3.8	− 3.6	1.9	0.4
Jan. 23	30	− 5.8	− 7.2	− 6.3	− 6.7	− 6.5	1.4	0.4
Jan. 30	31	− 4.2	− 5.8	− 4.5	− 4.9	− 4.7	1.7	0.4
Feb. 6	32	− 3.6	− 5.3	− 4.1	− 4.2	− 4.1	1.7	0.1
Feb. 13	33	− 3.3	− 5.0	− 3.6	− 4.1	− 3.8	1.7	0.4
Feb. 20	34	− 3.3	− 5.8	− 4.4	− 4.8	− 4.6	2.5	0.4
Feb. 27	35	− 3.3	− 3.9	− 3.4	− 3.5	− 3.45	0.6	0.1
Mar. 6	36	− 2.6	− 3.4	− 2.8	− 3.3	− 3.0	0.8	0.5
Mar. 13	37	3.9	− 2.8	0.0	− 1.0	− 0.5	6.7	1.0
Mar. 20	38	6.7	1.7	5.7	2.2	4.4	5.0	3.5
Mar. 27	39	8.3	− 0.5	4.7	2.9	3.7	8.8	1.8
Apr. 3	40	6.3	0.1	4.4	2.9	3.7	6.2	1.5
Apr. 10	41	12.8	5.3	10.0	9.1	9.8	7.5	0.9
Apr. 17	42	13.8	5.8	10.3	7.9	9.2	8.0	2.4
Apr. 24	43	12.5	3.9	8.2	5.8	7.0	8.6	2.4

Week Ending	Week No.	Abs. Max.	Abs. Min.	Mean Max.	Mean Min.	Mean Temp.	Extreme Range	Mean Daily Range
May 1	44	10.3	4.7	8.7	6.8	7.8	5.6	1.9
May 8	45	14.3	11.3	13.4	11.8	12.6	3.0	1.6
May 15	46	14.4	10.6	14.1	12.8	13.5	3.8	1.3
May 22	47	12.7	9.6	11.7	10.9	11.3	3.1	0.8
May 29	48	15.0	12.3	14.4	13.3	13.9	3.3	1.1
June 5	49	15.0	11.9	13.9	12.7	13.3	3.1	1.2
June 12	50	18.6	13.9	17.3	15.8	16.6	4.7	1.5
June 19	51	19.4	15.4	17.8	16.3	17.1	4.0	1.5
June 26	52	17.7	13.6	16.3	14.4	15.3	4.1	1.9

TABLE F

Evaporation Data, as Obtained from Porous Cup Atmometers

Station Number		1	2	3	4	5	6	7	8	9	10	11
Height (m)		1.0	0.15	1.5	1.5	2.5	0.15	0.15	6.0	10.0	12.0 15.0†	0.15
Date	Week	Average daily evaporation in cc. (reduced to standard)										
June 30		*	*	*	*	*	*	*				
July 2		11.1										
July 4	1	12.0	7.5	12.6	11.0	13.7	2.4	20.9				
July 11	2	13.6	8.5	14.3	14.3	14.9	7.6	20.7				
July 18	3	19.1	13.0	19.4	19.4	20.2	7.6	24.8	*			
July 25	4	14.8	9.7	15.3	15.3	16.2	10.2	24.9		*		
Aug. 1	5	13.9	9.5	15.2	14.6	15.1	8.6	‡	19.6	15.4		
Aug. 8	6	7.9	5.0	8.3	8.6	9.0	2.8		10.7	10.9	*	*
Aug. 15	7	6.6	4.7	7.7	7.8	7.8	2.6		9.7	10.8	12.2	8.8
Aug. 22	8	8.1	5.0	8.1	8.2	9.0	2.8		10.1	10.1	11.2	8.1
Aug. 29	9	4.6	2.8	4.6	4.8	5.7	1.3		7.7	6.9	6.3	6.2
Sept. 5	10	4.4	2.9	5.5	5.4	5.7	1.7		4.6	8.9	¶	6.5
Sept. 12	11	4.3	2.5	4.6	4.4	5.0	1.3			6.9	10.1	6.3
Sept. 19	12	5.0	3.9	5.9	6.0	6.6	1.6		6.7	7.7	13.9	5.1
Sept. 26	13	7.6	4.2	8.4	7.7	8.9	3.3		10.6	8.7	12.0	5.1
Oct. 3	14	7.0	4.8	8.8	7.9	8.6	3.3		9.3	9.2	14.1	6.3
Oct. 10	15	8.6	5.5	9.9	9.0	9.6	5.0		9.4	9.3	8.7	6.7
Oct. 17	16	11.6										
Oct. 24	17	15.2										
Oct. 31	18	11.0										
Nov. 7	19	13.6										
Nov. 14		frozen										
Apr. 17		*		*		*						

Station Number		1	2	3	4	5	6	7	8	9	10	11
Height (m)		1.0	0.15	1.5	1.5	2.5	0.15	0.15	6.0	10.0	12.0 15.0†	0.15
Date	Week	Average daily evaporation in cc. (reduced to standard)										
Apr. 24	43	12.6		12.3		11.7						
May 1	44	13.2	*	13.5		16.9						
May 8	45	17.3	11.5	17.3		20.2						
May 15	46	10.3	6.2	10.0		10.6						
May 22	47		3.4	7.5		8.0						
May 29	48	6.7	4.1	6.8		9.0						
June 5	49	10.6	5.9	10.7		13.3						
June 12	50		5.1	12.3		12.9						
June 19	51	12.3	7.2	12.7		14.7						
June 26	52	9.7	6.2	9.9		11.7						
July 3	53	11.5	7.1	11.3		14.0						

* Atmometer set up on this date. First reading includes evaporation beginning on this date.

† Atmometer changed to another tree at greater height on September 5.

‡ Atmometer stolen and observations at this station discontinued. See Station 11.

¶ Atmometer moved on this date to a height of 15 m. in another tree.

Location of Stations:

1—Top of instrument shelter.
2—On ground under shrubs.
3—South side of tree trunk.
4—North side of tree trunk.
5—Suspended under leafy maple branch.
6—In hollow stump.
7—At west edge of woods, short grass.
8—In tree.
9—In tree.
10—At level of tree tops, first at 12 m, later at 15 m.
11—At east edge of woods, tall grass.

TABLE G

Diurnal Variations in Light Intensity as Determined by the Macbeth Illuminometer
August 29-30, 1921

Time	Light Intensity in Meter-Candles		
	In Forest		In Forest Glade 1.25 m. above Ground Level
	Ground Level Under Herbage	1.25 m. above Ground Level	
Aug. 30			
7:20 a.m.			1140
7:50-8:10 a.m.	102	170	44940
9:00-9:10	155	355	77400
10:00-10:10	113	705	82220
12:30-12:40 p.m.		1170	96650
1:00	378		
Aug. 29			
2:40-3:10 p.m.	184	430	83660
4:05-4:25	107	210	
4:45-5:15	43	95	17680
5:50-5:55		14	2570
6:00-6:15			1570

TABLE H

Animal Population of the Lower Strata of University Woods, July 1921 to June 1922

Week Ending	Week Number	Soil Stratum		Leaf Stratum		Herb Stratum		Shrub Stratum		Total	Per Acre (thousands)	Per Hectare (thousands)
		Number of Samples	Average per Sample	Number of Samples	Average per Sample	Number of Samples	Average per Sample	Number of Samples	Average per Sample			
July 4	1	1	11	2	24.5	3	9	3	0.6	45.1	496	1218
July 11	2	1	16	1	20	6	13.5		2	51.5	566	1391
July 18	3	1	10	1	14	3	15	3	4.8	43.8	481	1183
July 25	4	1	15	2	5	3	8.4		4.2	32.6	359	980
Aug. 1	5	3	14.7	1	11		11.5		3.9	41.1	452	1109
Aug. 8	6	2	14.5	1	12	3	14	3	3.6	44.1	485	1191
Aug. 15	7											
Aug. 22	8	1	18	1	15	3	8	2	4.5	44.5	490	1201
Aug. 29	9	1	19	1	21	5	5.8	1	1	46.8	515	1264
Sept. 5	10		14.5		32	5	18.2	5	3.4	68.1	749	1839
Sept. 12	11	1	10	1	43	1	22	1	15	90	990	2437

Week Ending	Week Number	Soil Stratum		Leaf Stratum		Herb Stratum		Shrub Stratum		Total	Per Acre (thousands)	Per Hectare (thousands)
		Number of Samples	Average per Sample	Number of Samples	Average per Sample	Number of Samples	Average per Sample	Number of Samples	Average per Sample			
Sept. 19	12	1	17	1	24	1	30	1	15	86	946	2322
Sept. 26	13		15		60	2	54	2	78.5	207.5	2283	5603
Oct. 3	14		13	1	133	1	283	1	69	498	5480	13446
Oct. 10	15											
Oct. 17	16		7	1	49	1	128	1	24	208	2288	5616
Oct. 24	17											
Oct. 31	18	1	27	1	146	1	25	1	14	212	2352	5734
Nov. 7	19	1	12	1	125	1	23	1	5	169	1859	4773
Nov. 14	20		13	1	85		20		4.5	121.5	1337	3280
Nov. 21	21	1	14	1	54	1	16	1	4	82	902	2214
Nov. 28	22	1	1	1	33	1	37	1	9	93	1023	2511
Dec. 5	23	1	13	1	36	1	11	1	0	48	528	1296
Dec. 12	24	1	5	1	51	1	2	1	0	66	726	1782
Dec. 19	25	1	5	1	22	1	16	1	0	43	473	1161
Dec. 26	26											
Jan. 2	27	1	3	1	34	1	8	1	0	45	495	1215
Jan. 9	28	2	5.5	1	58	1	4	1	0	67.5	743	1823
Jan. 16	29	1	3	1	15	1	0	1	0	18	198	486
Jan. 23	30			1	22							
Jan. 30	31			1	22							
Feb. 6	32	1	7	1	90	1	3	1	0	100	1100	2700
Feb. 13	33	1	6	1	10	1	0	1	0	17	187	459
Feb. 20	34	1	7	1	16	1	0	1	0	23	258	621
Feb. 27	35	1	7	1	29	1	0	1	0	36	396	972
Mar. 6	36	1	10	1	7	1	4	1	0	21	231	567
Mar. 13	37	1	4	1	13	1	12	1	3	32	352	864
Mar. 20	38			1	23							
Mar. 27	39	1	14	1	74	1	6	1	1	95	1045	2565
Apr. 3	40	1	5	1	28	1	11	1	2	46	506	1242
Apr. 10	41	1	6	1	68	1	39	1	23	136	1496	3772
Apr. 17	42											
Apr. 24	43	1	0	1	34	1	3	1	163	200	2200	5400
May 1	44	1	13	1	39	1	51	1	398	501	5511	13527
May 8	45	1	1	1	26	1	400	1	64	491	5501	13257
May 15	46	1	22	1	9	1	52	1	51	134	1474	3618
May 22	47			1	9	1	4	1	63			
May 29	48	1	8	1	7	1	34	1	42	91	1001	2457
June 5	49	1	1	1	2	1.5	24	1.5	8	35	385	955
June 12	50	1	10	1	6	1	54	1	25	95	1045	2565
June 19	51	1	10	1	5	1	40	1	31	86	946	2322
June 26	52	1	0	1	0	1	55	1	32	87	957	2349
July 3	1	1	14	1	5	1	55	1	20	94	1034	2538

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